



INTERNATIONAL APPLIC

N PUBLISHED UNDER THE PATENT

OPERATION TREATY (PCT)

(51) International Patent Classification : A61K 47/48		A1	(11) International Publication Number: WO 96/36362
			(43) International Publication Date: 21 November 1996 (21.11.96)
(21) International Application Number: PCT/US96/07164 (22) International Filing Date: 16 May 1996 (16.05.96) (30) Priority Data: 08/441,979 16 May 1995 (16.05.95) US (71) Applicant: PRIZM PHARMACEUTICALS, INC. [US/US]; 11035 Roselle Street, San Diego, CA 92121 (US). (72) Inventors: SOSNOWSKI, Barbara, A.; 1013 Adella Avenue, Coronado, CA 92118 (US). BAIRD, J., Andrew; 5309 Via Papel, San Diego, CA 92122 (US). CHANDLER, Lois, A.; 1814 Blue Bonnet Place, Encinitas, CA 92024 (US). (74) Agents: NOTTENBURG, Carol et al.; Seed and Berry L.L.P., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: COMPOSITIONS CONTAINING NUCLEIC ACIDS AND LIGANDS FOR THERAPEUTIC TREATMENT

(57) Abstract

Preparations of conjugates of a receptor-binding internalized ligand and a cytocide-encoding agent and compositions containing such preparations are provided. The conjugates contain a polypeptide that is reactive with an FGF receptor, such as bFGF, or another heparin-binding growth factor, cytokine, or growth factor coupled to a nucleic acid binding domain. One or more linkers may be used in the conjugation. The linker is selected to increase the specificity, toxicity, solubility, serum stability, or intracellular availability, and promote nucleic acid condensation of the targeted moiety. The conjugates are complexed with a cytocide-encoding agent, such as DNA encoding saporin. Conjugates of a receptor-binding internalized ligand to a nucleic acid molecule are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France			UZ	Uzbekistan

DescriptionCOMPOSITIONS CONTAINING NUCLEIC
ACIDS AND LIGANDS FOR THERAPEUTIC TREATMENT

5

Technical Field

The present invention relates generally to the treatment of diseases, and more specifically, to the preparation and use of complexes containing receptor-binding internalized ligands NABD and cytocide-encoding agents to alter the function, gene
10 expression, or viability of a cell in a therapeutic manner.

Background of the Invention

A major goal of treatment of neoplastic diseases and hyperproliferative disorders is to ablate the abnormally growing cells while leaving normal cells
15 untouched. Various methods are under development for providing treatment, but none provide the requisite degree of specificity.

One method of treatment is to provide toxins. Immunotoxins and cytotoxins are protein conjugates of toxin molecules with either antibodies or factors which bind to receptors on target cells. Three major problems may limit the usefulness
20 of immunotoxins. First, the antibodies may react with more than one cell surface molecule, thereby effecting delivery to multiple cell types, possibly including normal cells. Second, even if the antibody is specific, the antibody reactive molecule may be present on normal cells. Third, the toxin molecule may be toxic to cells prior to delivery and internalization. Cytotoxins suffer from similar disadvantages of specificity
25 and toxicity. Another limitation in the therapeutic use of immunotoxins and cytotoxins is the relatively low ratio of therapeutic to toxic dosage. Additionally, it may be difficult to direct sufficient concentrations of the toxin into the cytoplasm and intracellular compartments in which the agent can exert its desired activity.

Given these limitations, cytotoxic therapy has been attempted using viral
30 vectors to deliver DNA encoding the toxins into cells. If eukaryotic viruses are used, such as the retroviruses currently in use, they may recombine with host DNA to produce infectious virus. Moreover, because retroviral vectors are often inactivated by the

complement system, use *in vivo* is limited. Retroviral vectors also lack specificity in delivery; receptors for most viral vectors are present on a large fraction, if not all, cells. Thus, infection with such a viral vector will infect normal as well as abnormal cells. Because of this general infection mechanism, it is not desirable for the viral vector to
5 directly encode a cytotoxic molecule.

While delivery of nucleic acids offers advantages over delivery of cytotoxic proteins such as reduced toxicity prior to internalization, there is a need for high specificity of delivery, which is currently unavailable with the present systems.

In view of the problems associated with gene therapy, there is a
10 compelling need for improved treatments which are more effective and are not associated with such disadvantages. The present invention exploits the use of conjugates which have increased specificity and deliver higher amounts of nucleic acids to targeted cells, while providing other related advantages.

15 Summary of the Invention

The present invention generally provides therapeutic compositions. In one aspect, the composition has the formula: receptor-binding internalized ligand—nucleic acid binding domain—cytocide-encoding agent. The receptor-binding internalized ligand is a polypeptide reactive with a cell surface receptor, the nucleic acid
20 binding domain binds to a nucleic acid, the cytocide-encoding agent is a nucleic acid molecule encoding a cytocide and which binds to the nucleic acid binding domain, and the composition binds to the cell surface receptor and internalizes the cytocide-encoding agent in cells bearing the receptor. In another aspect, the composition has the formula: receptor-binding internalized ligand-nucleic acid binding domain-prodrug-encoding
25 agent.

In certain embodiments, the receptor-binding internalized ligand is a polypeptide reactive with an FGF receptor, VEGF receptor, HBEGF receptor, or a cytokine. In other embodiments, the cytocide-encoding agent encodes a protein that inhibits protein synthesis and is preferably a ribosome inactivating protein, most
30 preferably saporin. The protein is gelonin or diphtheria toxin in other embodiments. In other embodiments, the prodrug-encoding agent encodes HSV-thymidine kinase.

The nucleic acid binding domain is poly-L-lysine in one embodiment. In other embodiments, the nucleic acid binding domain is a transcription factor selected from the group consisting of helix-turn-helix motif proteins, homeodomain proteins, zinc finger motif proteins, steroid receptor proteins, leucine zipper motif proteins, helix-loop-helix motif proteins, and β -sheet motif proteins. In other embodiments, the nucleic acid binding domain binds nonspecifically to nucleic acids and is selected from the group consisting of poly-L-lysine, protamine, histone and spermine. In a preferred embodiment, the nucleic acid binding domain binds the coding region of a ribosome inactivating protein such as saporin. In another preferred embodiment, FGF is conjugated to poly-L-lysine.

In yet other embodiments, the cytocide-encoding agent contains a tissue-specific promoter, such as alpha-crystalline, gamma-crystalline, α -fetoprotein, CEA, prostate-specific antigen, *erbB-2*, tyrosinase, α -actin, *c-myc*, VEGF receptor, FGF receptor or cyclin D.

In another aspect, the composition also contains a linker. In various embodiments, the linker increases the flexibility of the conjugate and is $(\text{Gly}_m\text{Ser}_p)_n$, $(\text{Ala Ala Pro Ala})_n$, wherein n is 1 to 6, m is 1 to 6 and p is 1 to 4, or the linker is a disulfide bond.

In yet another aspect, the composition has the formula: receptor-binding internalized ligand-cytocide encoding agent-nucleic acid binding domain, wherein the receptor-binding internalized ligand is conjugated to the cytocide-encoding agent, which is bound to the nucleic acid binding domain to form a complex.

In other aspects, the invention provides methods for preventing excessive cell proliferation in the anterior eye following surgery, treating corneal clouding following excimer laser surgery, preventing closure of a trabeculectomy, preventing pterygii recurrence, treating hyperproliferative diseases in the back of the eye, such as macular degeneration, diabetic retinopathy and proliferative vitreal retinopathy, treating smooth muscle cell hyperplasia after a wound healing response to a procedure,

e.g., vein grafting, endarterectomies and arteriovenous shunts and treating cancer. In these aspects, an effective amount of the compositions described above are administered.

5 Brief Description of the Drawings

Figure 1 is a photograph of an SDS-PAGE of FGF2-K152 under non-reducing (left) and reducing (right) conditions. Lane 1, FGF2-K152; lane 2, FGF2; lane 3, FGF2-K152; lane 4, FGF2. The open arrow identifies material unable to enter the gel. The closed arrow identifies a protein band corresponding to FGF2.

10 Figure 2 is a graph depicting the proliferation of bovine aortic endothelial cells in response to FGF2 (closed box) and FGF2-K152 (open circle) conjugate.

Figure 3 is a photograph of a gel showing the effects of various lengths of poly-L-lysine on the ability to interact with DNA. Thirty-five ng of labeled DNA
15 were added to increasing concentrations of either FGF2 or FGF2-K: lanes 1, 0 ng; lanes 2, 0.1 ng; lanes 3, 1 ng; lanes 4, 10 ng; lanes 5, 20 ng; lanes 6, 35 ng; lanes 7, 100 ng. Panel A: FGF2; panel B, FGF2-K152; panel C, FGF2-K13; panel D, FGF2-K84; panel E, EGF2-K267; panel F, FGF2-K39. The lengths of the digested DNA are indicated.

20 Figure 4 is a chart depicting the activity of β -gal following transfection of FGF2/poly-L-lysine/DNA β -gal into COS cells. Lane 1, 10:1 (w/w) ratio of FGF2/poly-L-lysine conjugate to DNA; lane 2, 5:1 ratio; lane 3, 2:1 ratio; lane 4, 1:1 ratio; lane 5, 0.5:1 ratio. The five bars, from left to right, are FGF2, FGF2-K13, FGF2-K39, FGF2-K84, and FGF2-K152.

25 Figure 5 are photographs of toroid format observed by electron microscopy. The upper panel shows an example of a toroid; the lower panel shows an incomplete toroid.

Figure 6 is a graph depicting proliferation of bovine aortic-endothelial cells. In the upper panel, cells were treated with FGF2-K152-DNA; in the lower panel,
30 cells were treated with a mixture of FGF2, K152, and DNA.

Figure 7A is a graph displaying β -gal activity after transfection of FGF2/poly-L-lysine/pSV β -gal into COS cells (lane 1), B16 cells (lane 2), NIH 3T3 cells (lane 3), and BHK cells (lane 4).

Figure 7B is a graph depicting β -gal expression in COS cells, pSV β -gal (lanes 1, 3) or pNASS β -gal (lanes 2, 4) were incubated with (lanes 1, 2) or without (lanes 3, 4) FGF2-K84 and the complexes incubated on COS cells for 48 hrs.

Figure 7C is a graph showing activity of β -gal activity at various times following transfection with either plasmid alone or with complexes of FGF2/K84/pSV β -gal. - Δ -, DNA alone; - \blacksquare -, FGF2-K84-DNA.

Figure 7D is a graph showing β -gal activity after transfection of various concentrations of FGF2/K84/pSV β -gal. Lane 1, 0 μ g; lane 2, 0.1 μ g; lane 3, 1 μ g; lane 4, 5 μ g; lane 5, 10 μ g.

Figure 8A is a graph showing β -gal activity in COS cells following transfection of FGF2-K84-pSV β -gal (lane 1), FGF2+K84+pSV β -gal (lane 2), FGF2+pSV β -gal (lane 3), K84+pSV β -gal (lane 4); pSV β -gal (lane 5), FGF2-K84 (lane 6), FGF2 (lane 7) and K84 (lane 8).

Figure 8B is a graph showing completion for cell bindings. Lane 1, FGF2-K84-pSV β -gal complex transfected into COS cells; lane 2, FGF2-K84-pSV β -gal plus 100 μ g FGF2; lane 3, no complex.

Figure 8C is a graph showing the attenuation of β -gal activity upon the addition of heparin during transfection. Lane 1, FGF2-K84-pSV β -gal+10 μ g heparin; lane 2, FGF2-K84-pSV β -gal; lane 3, heparin alone; lane 4, pSV β -gal alone.

Figure 8D is a graph showing ligand targeting of DNA, pSV β -gal DNA alone (lane 1), FGF2-K84 (lane 2), histone H1-K84 (lane 3) and cytochrome C-K84 (lane 4) were condensed with pSV β -gal DNA and added to BHK cells. β -gal activity was measured 48 hr later.

Figure 9A is a graph showing the effect of chloroquine on β -gal expression, pSV β -gal and FGF2-K84 were mixed in the absence (lane 1) or presence (lane 2) of 100 μ M chloroquine and incubated for 1 hr at room temperature prior to addition of the complexes to COS cells. Lane 3, chloroquine alone; lane 4, DNA alone.

Figure 9B is a graph showing the effect of endosome disruptive peptide on β -gal expression. Lane 1, control; lane 2, FGF2-K84-pSV β -gal; lane 3, FGF2-K84-pSV β -gal+EDP.

5 Figure 9C are photographs of cells stained for β -gal activity following transfection of COS cells with (right panel) or without (left panel) endosome disruptive peptide and FGF2-K84-pSV β -gal.

Figure 10 is a photograph of a fluorograph analyzing cell-free translation products. Lane 1, no RNA; lane 2, saporin RNA; lane 3, luciferase RNA; lane 4, saporin RNA and luciferase RNA; lane 5, saporin RNA followed 30 min later with
10 luciferase RNA.

Figure 11 is a graph depicting direct cytotoxicity of cells transfected by a CaPO_4 with an expression vector encoding saporin. Lane 1, mock transfection; lane 2, transfection with pSV β -gal; lane 3, transfection with saporin-containing vector.

Figure 12 is a pair of graphs showing cytotoxicity of cells transfected
15 with FGF2-K84-pSVSAP. Left panel, BHK21 cells; right panel, NIH 3T3 cells. Lane 1, FGF2-K84-pSV β -gal; lane 2, FGF2-K84-pSVSAP.

Figure 13A is a graph showing β -gal activity with an endosome disruptive peptide in the complex.

Figure 13B is a graph showing β -gal activity with an endosome
20 disruptive peptide in the complex.

Figure 13C is a graph showing β -gal activity with an endosome disruptive peptide in the complex.

Detailed Description of the Invention

25 Definitions

All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto.

The "amino acids," which occur in the various amino acid sequences appearing herein, are identified according to their well known, three letter or one letter

abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard single letter designations used routinely in the art.

As used herein, to "bind to a receptor" refers to the ability of a ligand to specifically recognize and detectably bind to such receptors, as assayed by standard *in vitro* assays. For example, as used herein, binding measures the capacity of a VEGF conjugate, VEGF monomer, or VEGF dimer to recognize a VEGF receptor on a vascular endothelial cell, such as an aortic vascular endothelial cell line, using a procedure substantially as described in Moscatelli, *J. Cell Physiol.* 131:123-130, 1987.

As used herein, "biological activity" refers to the *in vivo* activities of a compound or physiological responses that result upon *in vivo* administration of a compound, composition or other mixture. Biological activity thus encompasses therapeutic effects and pharmaceutical activity of such compounds, compositions and mixtures. Such biological activity may be defined with reference to particular *in vitro* activities as measured in a defined assay. For example, reference herein to the biological activity of FGF, or fragments of FGF, refers to the ability of FGF to bind to cells bearing FGF receptors and internalize a linked agent. Such activity is typically assessed *in vitro* by linking the FGF to a cytotoxic agent, such as saporin, contacting cells bearing FGF receptors, such as fibroblasts, with the conjugate and assessing cell proliferation or growth. *In vivo* activity may be determined using recognized animal models, such as the mouse xenograft model for anti-tumor activity (*see, e.g.*, Beitz et al., *Cancer Research* 52:227-230, 1992; Houghton et al., *Cancer Res.* 42:535-539, 1982; Bogden et al., *Cancer (Philadelphia)* 48:10-20, 1981; Hoogenhout et al., *Int. J. Radiat. Oncol., Biol. Phys.* 9:871-879, 1983; Stastny et al., *Cancer Res.* 53:5740-5744, 1993).

As used herein, reference to the "biological activity of a cytocide-encoding agent," such as DNA encoding saporin, refers to the ability of such agent to interfere with the metabolism of the cell by inhibiting protein synthesis. Such biological or cytotoxic activity may be assayed by any method known to those of skill in the art including, but not limited to, *in vitro* assays that measure protein synthesis and *in vivo* assays that assess cytotoxicity by measuring the effect of a test compound on

cell proliferation or on protein synthesis. Assays that assess cytotoxicity in targeted cells are particularly preferred.

As used herein, a "conjugate" refers to a molecule that contains at least one receptor-internalized binding ligand and at least one nucleic acid binding domain
5 that are linked directly or via a linker and that are produced by chemical coupling methods or by recombinant expression of chimeric DNA molecules to produce fusion proteins.

A "cytocide-encoding agent" is a nucleic acid molecule that encodes a protein that inhibits protein synthesis. Such a protein may act by cleaving rRNA or
10 ribonucloprotein, inhibiting an elongation factor, cleaving mRNA, or other mechanism that reduces protein synthesis to a level such that the cell cannot survive. The cytocide-encoding agent may contain additional elements besides the cytocide gene. Such elements include a promoter, enhancer, splice sites, transcription terminator, poly(A) signal sequence, bacterial or mammalian origins of replication, selection markers, and
15 the like.

As used herein, the term "cytotoxic agent" refers to a molecule capable of inhibiting cell function. The agent may inhibit proliferation or may be toxic to cells. A variety of cytotoxic agents can be used and include those that inhibit protein synthesis and those that inhibit expression of certain genes essential for cellular growth
20 or survival. Cytotoxic agents include those that result in cell death and those that inhibit cell growth, proliferation and/or differentiation.

As used herein, cytotoxic agents include, but are not limited to, saporin, the ricins, abrin and other ribosome inactivating proteins (RIPs), aquatic-derived cytotoxins, *Pseudomonas exotoxin*, inhibitors of DNA, RNA or protein synthesis, such
25 as antisense nucleic acids, other metabolic inhibitors, such as DNA cleaving molecules, prodrugs, such as thymidine kinase from HSV and bacterial cytosine deaminase, and light activated porphyrin. While saporin is the preferred RIP, other suitable RIPs include ricin, ricin A chain, maize RIP, gelonin, diphtheria toxin, diphtheria toxin A chain, trichosanthin, tritin, pokeweed antiviral protein (PAP), mirabilis antiviral protein
30 (MAP), Dianthins 32 and 30, abrin, monordin, bryodin, shiga, a catalytic inhibitor of

protein biosynthesis from cucumber seeds (*see, e.g.,* WO 93/24620), *Pseudomonas* *exotoxin*, biologically active fragments of cytotoxins and others known to those of skill in this art. Suitable cytotoxic agents also include cytotoxic molecules that inhibit cellular metabolic processes, including transcription, translation, biosynthetic or
5 degradative pathways, DNA synthesis, and other such processes that kill cells or inhibit cell proliferation.

"Heparin-binding growth factor" refers to any member of a family of heparin-binding growth factor proteins, in which at least one member of the family binds heparin. Preferred growth factors in this regard include FGF, VEGF, and
10 HBEGF. Such growth factors encompass isoforms, peptide fragments derived from a family member, splice variants, and single or multiple exons, some forms of which may not bind heparin.

As used herein, to "hybridize" under conditions of a specified stringency is used to describe the stability of hybrids formed between two single-stranded nucleic
15 acid molecules. Stringency of hybridization is typically expressed in conditions of ionic strength and temperature at which such hybrids are annealed and washed. Typically high, medium and low stringency encompass the following conditions or equivalent conditions thereto:

- 1) high stringency: 0.1 x SSPE or SSC, 0.1% SDS, 65°C
- 20 2) medium stringency: 0.2 x SSPE or SSC, 0.1% SDS, 50°C
- 3) low stringency: 1.0 x SSPE or SSC, 0.1% SDS, 50°C.

"Nucleic acid binding domain" (NABD) refers to a molecule, usually a protein, polypeptide, or peptide (but may also be a polycation) that binds nucleic acids, such as DNA or RNA. The NABD may bind to single or double strands of RNA or
25 DNA or mixed RNA/DNA hybrids. The nucleic acid binding domain may bind to a specific sequence or bind irrespective of the sequence.

As used herein, "nucleic acids" refer to RNA or DNA that are intended for internalization into a cell and includes, but are not limited to, DNA encoding a therapeutic protein, DNA encoding a cytotoxic protein, DNA encoding a prodrug, DNA
30 encoding a cytocide, the complement of these DNAs, an antisense nucleic acid and

other such molecules. Reference to nucleic acids includes duplex DNA, single-stranded DNA, RNA in any form, including triplex, duplex or single-stranded RNA, anti-sense RNA, polynucleotides, oligonucleotides, single nucleotides, chimeras, and derivatives thereof.

5 Nucleic acids may be composed of the well-known deoxyribonucleotides and ribonucleotides composed of the bases adenosine, cytosine, guanine, thymidine, and uridine. As well, various other nucleotide derivatives and non-phosphate backbones or phosphate-derivative backbones may be used. For example, because normal phosphodiester oligonucleotides (referred to as PO oligonucleotides) are sensitive to
10 DNA- and RNA-specific nucleases, several resistant types of oligonucleotides have been developed in which the phosphate group has been altered to a phosphotriester, methylphosphonate, or phosphorothioate (*see* U.S. Patent No. 5,218,088).

As used herein, "operative linkage" or operative association of heterologous DNA to regulatory and effector sequences of nucleotides, such as
15 promoters, enhancers, transcriptional and translational stop sites, refers to the functional relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically
20 recognizes, binds to and transcribes the DNA in reading frame.

As used herein, the term "polypeptide reactive with an FGF receptor" refers to any polypeptide that specifically interacts with an FGF receptor, preferably the high-affinity FGF receptor and that is transported into the cell by virtue of its interaction with the FGF receptor. Polypeptides reactive with an FGF receptor are also
25 called FGF proteins. Such polypeptides include, but are not limited to, FGF-1 to FGF-9. For example, bFGF (FGF-2) should be generally understood to refer to polypeptides having substantially the same amino acid sequences and receptor-targeting activity as that of bovine bFGF or human bFGF. It is understood that differences in amino acid sequences can occur among FGFs of different species as well as among FGFs from
30 individual organisms or species. In addition, chimeras or hybrids of any of FGF-1

through FGF-9, or FGFs that have deletions (*see, e.g.*, PCT Application No. WO 90/02800), insertions or substitutions of amino acids are within the scope of FGF proteins, as long as the resulting peptide or protein specifically interacts with an FGF receptor and is internalized by virtue of this interaction.

5 As used herein, a "prodrug" is a compound that metabolizes or otherwise converts an inactive, nontoxic compound to a biologically, pharmaceutically, therapeutically, of toxic active form of the compound. A prodrug may also be a pharmaceutically inactive compound that is modified upon administration to yield an active compound through metabolic or other processes. The prodrug may alter the
10 metabolic stability or the transport characteristics of a drug, mask side effects or toxicity, improve or alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design inactive forms of the compound (*see, e.g.*, Nogrady, *Medicinal Chemistry A Biochemical*
15 *Approach*, Oxford University Press, New York, pages 388-392, 1985).

 As used herein, "receptor-binding internalized ligand" or "ligand" refers to any peptide, polypeptide, protein or non-protein, such as a peptidomimetic, that is capable of binding to a cell-surface molecule and is internalized. Within the context of this invention, the receptor-binding internalized ligand is conjugated to a nucleic acid
20 binding domain, either as a fusion protein or through chemical conjugation, and is used to deliver a cytocide-encoding or pro-drug encoding agent to a cell. In one aspect, the ligand is directly conjugated to a nucleic acid molecule, which may be further complexed with a nucleic acid binding domain. Such ligands include growth factors, cytokines, antibodies or fragments thereof, hormones, and the like.

25 As used herein, "saporin" (abbreviated herein as SAP) refers to polypeptides that are isolated from the leaves or seeds of *Saponaria officinalis*, as well as modified forms that have amino acid substitutions, deletions, insertions or additions, which still express substantial ribosome inactivating activity. Purified preparations of saporin are frequently observed to include several molecular isoforms of the protein. It
30 is understood that differences in amino acid sequences can occur in saporin from

different species as well as between saporin molecules from individual organisms of the same species. Saporin for use herein may be purified from leaves, chemically synthesized, or synthesized by expression of DNA encoding a saporin polypeptide.

As used herein, a "targeted agent" is a nucleic acid molecule that is
5 intended for internalization by complexing or linkage to a receptor-binding internalized ligand, and nucleic acid binding domain, and that upon internalization in some manner alters or affects cellular metabolism, growth, activity, viability or other property or characteristic of the cell.

As used herein, a "therapeutic nucleic acid" describes any nucleic acid
10 molecule used in the context of the invention that modifies gene transcription or translation. This term also includes nucleic acids that bind to sites on proteins. It includes, but is not limited to, the following types of nucleic acids: nucleic acids encoding a protein, antisense RNA, DNA intended to form triplex molecules, extracellular protein binding oligonucleotides, and small nucleotide molecules. A
15 therapeutic nucleic acid may be used to effect genetic therapy by serving as a replacement for a defective gene, by encoding a therapeutic product, such as TNF, or by encoding a cytotoxic molecule, especially an enzyme, such as saporin. The therapeutic nucleic acid may encode all or a portion of a gene, and may function by recombining with DNA already present in a cell, thereby replacing a defective portion of a gene. It
20 may also encode a portion of a protein and exert its effect by virtue of co-suppression of a gene product.

PREPARATION OF RECEPTOR-BINDING INTERNALIZED LIGAND, NUCLEIC ACID BINDING DOMAIN AND CYTOTOXIC-ENCODING AGENT COMPLEXES

As noted above, the present invention provides cytotoxic-encoding agents
25 complexed with a conjugate of a receptor-binding internalized ligand and a nucleic acid binding domain. Upon binding to an appropriate receptor, the complex is internalized by the cell and is trafficked through the cell via the endosomal compartment, where at least a portion of the complex may be cleaved.

A. Receptor-binding internalized ligands

As noted above, receptor-binding internalized ligands are used to deliver a cytocide-encoding agent to a cell expressing an appropriate receptor on its cell surface. Numerous molecules that bind specific receptors have been identified and are suitable for use in the present invention. Such molecules include growth factors, cytokines, and antibodies. Many growth factors and families of growth factors share structural and functional features and may be used in the present invention. One such family of growth factors specifically binds to heparin. The ability of heparin-binding growth factors to interact with heparin appears in general to be a reflection of a physiologically more relevant interaction occurring *in vivo* between these factors and heparin sulfate proteoglycan molecules, which are found on the surface of cells and in extracellular matrix. Heparin-binding growth factors include the fibroblast growth factors FGF-1 through FGF-9, vascular endothelial growth factor (VEGF), and heparin binding-epidermal growth factor (HBEGF). Antibodies that are specific to cell surface molecules expressed by a selected cell type are readily generated as monoclonals or polyclonal antisera. Many such antibodies are available (*e.g.*, American Type Culture Collection, Rockville, MD). Other growth factors, such as PDGF (platelet-derived growth factor), EGF (epidermal growth factor), TGF- α (tumor growth factor), TGF- β , IGF-I (insulin-like growth factor), and IGF-II also bind to specific identified receptors on cell surfaces and may be used in the present invention. Cytokines, including interleukins, CSFs (colony stimulating factors), and interferons, have specific receptors, which are mostly found on hematopoietic cells, and may be used as described herein. These ligands are discussed in more detail below.

Fragments of these ligands may be used within the present invention, so long as the fragment retains the ability to bind to the appropriate cell surface molecule. Likewise, ligands with substitutions or other alterations, but which retain binding ability, may also be used.

1. Fibroblast growth factors

One family of growth factors that has a broad spectrum of activities is the fibroblast growth factor (FGF) family. These proteins share the ability to bind to

heparin, induce intracellular receptor-mediated tyrosine phosphorylation and the expression of the *c-fos* mRNA transcript, and stimulate DNA synthesis and cell proliferation. This family of proteins includes FGFs designated FGF-1 (acidic FGF (aFGF)), FGF-2 (basic FGF (bFGF)), FGF-3 (int-2) (*see, e.g.*, Moore et al., *EMBO J.* 5:919-924, 1986), FGF-4 (hst-1/K-FGF) (*see, e.g.*, Sakamoto et al., *Proc. Natl. Acad. Sci. USA* 86:1836-1840, 1986; U.S. Patent No. 5,126,323), FGF-5 (*see, e.g.*, U.S. Patent No. 5,155,217), FGF-6 (hst-2) (*see, e.g.*, published European Application EP 0 488 196 A2; Uda et al., *Oncogene* 7:303-309, 1992), FGF-7 (keratinocyte growth factor) (KGF) (*see, e.g.*, Finch et al., *Science* 245:752-755, 1985; Rubin et al., *Proc. Natl. Acad. Sci. USA* 86:802-806, 1989; and International Application WO 90/08771), FGF-8 (*see, e.g.*, Tanaka et al., *Proc. Natl. Acad. Sci. USA* 89:8528-8532, 1992); and FGF-9 (*see, Miyamoto et al., Mol. Cell. Biol.* 13:4251-4259, 1993).

DNA encoding FGF peptides and/or the amino acid sequences of FGFs are known to those of skill in the art. DNA encoding an FGF may be prepared synthetically based on a known amino acid or DNA sequence, isolated using methods known to those of skill in the art, or obtained from commercial or other sources. DNA encoding virtually all of the FGF family of peptides is known. For example, DNA encoding human FGF-1 (Jaye et al., *Science* 233:541-545, 1986; U.S. Patent No. 5,223,483), bovine FGF-2 (Abraham et al., *Science* 233:545-548, 1986; Esch et al., *Proc. Natl. Acad. Sci. USA* 82:6507-6511, 1985; and U.S. Patent No. 4,956,455), human FGF-2 (Abraham et al., *EMBO J.* 5:2523-2528, 1986; U.S. Patent No. 4,994,559; U.S. Patent No. 5,155,214; EP 470,183B; and Abraham et al., *Quant. Biol.* 51:657-668, 1986) and rat FGF-2 (*see* Shimasaki et al., *Biochem. Biophys. Res. Comm.*, 1988, and Kurokawa et al., *Nucleic Acids Res.* 16:5201, 1988), FGF-3, FGF-6, FGF-7 and FGF-9 are known (*see also* U.S. Patent No. 5,155,214; U.S. Patent No. 4,956,455; U.S. Patent No. 5,026,839; U.S. Patent No. 4,994,559, EP 0,488,196 A2, DNASTAR, EMBL or GenBank databases, and references discussed above and below). DNA encoding an FGF may be produced from any of the preceding DNA fragments by substitution of degenerate codons. It is understood that once the complete amino acid sequence of a peptide, such as an FGF peptide, and the DNA

fragment encoding such peptide are available to those of skill in this art, it is routine to substitute degenerate codons and produce any of the possible DNA fragments that encode such peptide. It is also generally possible to synthesize DNA encoding such peptide based on the amino acid sequence.

5 Thus, as used herein, "FGF" refers to polypeptides having amino acid sequences of native FGF proteins, as well as modified sequences, having amino acid substitutions, deletions, insertions or additions in the native protein but retaining the ability to bind to FGF receptors and to be internalized. It is understood that differences in amino acid sequences can occur among FGFs of different species as well as among
10 FGFs from individual organisms or species.

Reference to FGFs is intended to encompass proteins isolated from natural sources as well as those made synthetically, as by recombinant means or possibly by chemical synthesis. FGF also encompasses muteins that possess the ability to bind to FGF-receptor expressing cells. Such muteins include, but are not limited to,
15 those produced by replacing one or more of the cysteines with serine as described herein or that have any other amino acids deleted or replaced as long as the resulting protein has the ability to bind to FGF-receptor bearing cells and internalize the linked targeted agent. Typically, such muteins will have conservative amino acid changes, such as those set forth below in Table 1. DNA encoding such muteins will, unless
20 modified by replacement of degenerate codons, hybridize under conditions of at least low stringency to native DNA sequence encoding the starting FGF.

Acidic and basic FGF are about 55% identical at the amino acid level and are highly conserved among species. The other members of the FGF family have a high degree of amino acid sequence similarities and common physical and biological
25 properties with FGF-1 and FGF-2, including the ability to bind to one or more FGF receptors. Basic FGF, int-2, hst-1/K-FGF, FGF-5, hst-2/FGF-6 and FGF-8 may have oncogenic potential; bFGF is expressed in melanomas, int-2 is expressed in mammary tumor virus and hst-1/K-FGF is expressed in angiogenic tumors. Acidic FGF, bFGF, KGF and FGF-9 are expressed in normal cells and tissues.

FGFs exhibit a mitogenic effect on a wide variety of mesenchymal, endocrine and neural cells and are also important in differentiation and development. Of particular interest is their stimulatory effect on collateral vascularization and angiogenesis. In some instances, FGF-induced mitogenic stimulation may be detrimental. For example, cell proliferation and angiogenesis are an integral aspect of tumor growth. Members of the FGF family, including bFGF, are thought to play a pathophysiological role, for example, in tumor development, rheumatoid arthritis, proliferative diabetic retinopathies and other complications of diabetes.

The effects of FGFs are mediated by high affinity receptor tyrosine kinases present on the cell surface of FGF-responsive cells (*see, e.g.*, PCT WO 91/00916, WO 90/05522, PCT WO 92/12948; Imamura et al., *Biochem. Biophys. Res. Comm.* 155:583-590, 1988; Huang et al., *J. Biol. Chem.* 261:9568-9571, 1986; Partanen et al., *EMBO J.* 10:1347, 1991; and Moscatelli, *J. Cell. Physiol.* 131:123, 1987). Lower affinity receptors also appear to play a role in mediating FGF activities. The high affinity receptor proteins are single chain polypeptides with molecular weights ranging from 110 to 150 kD, depending on cell type that constitute a family of structurally related FGF receptors. Four FGF receptor genes have been identified, and three of these genes generate multiple mRNA transcripts via alternative splicing of the primary transcript.

20

2. Vascular endothelial growth factors

Vascular endothelial growth factors (VEGFs) were identified by their ability to directly stimulate endothelial cell growth, but do not appear to have mitogenic effects on other types of cells. VEGFs also cause a rapid and reversible increase in blood vessel permeability. The members of this family have been referred to variously as vascular endothelial growth factor (VEGF), vascular permeability factor (VPF) and vasculotropin (*see, e.g.*, Plouet et al., *EMBO J.* 8:3801-3806, 1989). Herein, they are collectively referred to as VEGF.

VEGF was originally isolated from a guinea pig hepatocarcinoma cell line, line 10 (*see, e.g.*, U.S. Patent No. 4,456,550), and has subsequently been identified in humans and in normal cells. It is expressed during normal development and in

certain normal adult organs. Purified VEGF is a basic, heparin-binding, homodimeric glycoprotein that is heat-stable, acid-stable and may be inactivated by reducing agents.

DNA sequences encoding VEGF and methods to isolate these sequences may be found primarily in U.S. Patent No. 5,240,848, U.S. Patent No. 5,332,671, U.S. Patent No. 5,219,739, U.S. Patent No. 5,194,596, and Houch et al., *Mol. Endocrin.* 5:180, 1991. As used herein, "DNA encoding a VEGF peptide or polypeptide" refers to any of the DNA fragments set forth herein as coding such peptides, to any such DNA fragments known to those of skill in the art, any DNA fragment that encodes a VEGF that binds to a VEGF receptor and is internalized thereby. VEGF DNA may be isolated from a human cell library, for example, using any of the preceding DNA fragments as a probe or any DNA fragment that encodes any of the VEGF peptides set forth in SEQ ID NOs. 1-4. It is understood that once the complete amino acid sequence of a peptide, such as a VEGF peptide, and the DNA fragment encoding such peptide are available to those of skill in this art, it is routine to substitute degenerate codons and produce any of the possible DNA fragments that encode such peptide. It is also generally possible to synthesize DNA encoding such peptide based on the amino acid sequence.

VEGF family members arise from a single gene organized as eight exons and spanning approximately 14 kb in the human genome. Four molecular species of VEGF result from alternative splicing of mRNA and contain 121, 165, 189 and 206 amino acids. The four species have similar biological activities, but differ markedly in their secretion patterns. The predominant isoform secreted by a variety of normal and transformed cells is VEGF₁₆₅. Transcripts encoding VEGF₁₂₁ and VEGF₁₈₉ are detectable in most cells and tissues that express the VEGF gene. In contrast, VEGF₂₀₆ is less abundant and has been identified only in a human fetal liver cDNA library. VEGF₁₂₁ is a weakly acidic polypeptide that lacks the heparin binding domain and, consequently, does not bind to heparin. VEGF₁₈₉ and VEGF₂₀₆ are more basic than VEGF₁₆₅ and bind to heparin with greater affinity. Although not every identified VEGF isoform binds heparin, all isoforms are considered to be heparin-binding growth factors within the context of this invention.

The secreted isoforms, VEGF₁₂₁ and VEGF₁₆₅ are preferred VEGF proteins. The longer isoforms, VEGF₁₈₉ and VEGF₂₀₆, are almost completely bound to the extracellular matrix and need to be released by an agent, such as suramin, heparin or heparinase, or plasmin. Other preferred VEGF proteins contain various combinations of VEGF exons, such that the protein still binds VEGF receptor and is internalized. It is not necessary that a VEGF protein used in the context of this invention either retain any of its *in vivo* biological activities, such as stimulating endothelial cell growth, or bind heparin. It is only necessary that the VEGF protein or fragment thereof bind the VEGF receptor and be internalized into the cell bearing the receptor. However, it may be desirable in certain contexts for VEGF to manifest certain of its biological activities. For example, if VEGF is used as a carrier for DNA encoding a molecule useful in wound healing, it would be desirable that VEGF exhibit vessel permeability activity and promotion of fibroblast migration and angiogenesis. It will be apparent from the teachings provided within the subject application which of the activities of VEGF are desirable to maintain.

VEGF promotes an array of responses in endothelium, including blood vessel hyperpermeability, endothelial cell growth, angiogenesis, and enhanced glucose transport. VEGF stimulates the growth of endothelial cells from a variety of sources (including brain capillaries, fetal and adult aortas, and umbilical veins) at low concentrations, but is reported to have no effect on the growth of vascular smooth muscle cells, adrenal cortex cells, keratinocytes, lens epithelial cells, or BHK-21 fibroblasts. VEGF also is a potent polypeptide regulator of blood vessel function; it causes a rapid but transient increase in microvascular permeability without causing endothelial cell damage or mast cell degranulation, and its action is not blocked by antihistamines. VEGF has also been reported to induce monocyte migration and activation and has been implicated as a tumor angiogenesis factor in some human gliomas. Also, VEGF is a chemoattractant for monocytes and VEGF has been shown to enhance the activity of the inflammatory mediator tumor necrosis factor (TNF).

Quiescent and proliferating endothelial cells display high-affinity binding to VEGF, and endothelial cell responses to VEGF appear to be mediated by

high affinity cell surface receptors (*see, e.g.*, International Application WO 92/14748, which is based on U.S. Applications Serial No. 08/657,236, de Vries et al., *Science* 255:989-91, 1992; Terman et al., *Biochem. Biophys. Res. Commun.* 187:1579-1586, 1992; Kendall et al., *Proc. Natl. Acad. Sci. USA* 90:10705-10709, 1993; and Peters et al., *Proc. Natl. Acad. Sci. USA* 90:8915-8919, 1993). Two tyrosine kinases have been identified as VEGF receptors. The first, known as fms-like tyrosine kinase or FLT is a receptor tyrosine kinase that is specific for VEGF. In adult and embryonic tissues, expression of FLT mRNA is localized to the endothelium and to populations of cells that give rise to endothelium. The second receptor, KDR (human kinase insert domain-containing receptor), and its mouse homologue FLK-1, are closely related to FLT. The KDR/FLK-1 receptor is expressed in endothelium during the fetal growth stage, during earlier embryonic development, and in adult tissues. In addition, messenger RNA encoding FLT and KDR have been identified in tumor blood vessels and specifically by endothelial cells of blood vessels supplying glioblastomas. Similarly, FLT and KDR mRNAs are upregulated in tumor blood vessels in invasive human colon adenocarcinoma, but not in the blood vessels of adjacent normal tissues.

3. Heparin-binding epidermal growth factors

Several new mitogens in the epidermal growth factor protein family have recently been identified that display the ability to bind the glycosaminoglycan heparin. Among these is the mitogen known as heparin-binding EGF-like growth factor (HBEGF), which elutes from heparin-Sepharose™ columns at about 1.0 - 1.2 M NaCl and which was first identified as a secreted product of cultured human monocytes, macrophages, and the macrophage-like U-937 cell line (Higashiyama et al., *Science* 251:936-939, 1991; Besner et al., *Cell Regul.* 1:811-19, 1990). HBEGF has been shown to interact with the same high affinity receptors as EGF on bovine aortic smooth muscle cells and human A431 epidermoid carcinoma cells (Higashiyama, *Science* 251:936-939, 1991).

HBEGFs exhibit a mitogenic effect on a wide variety of cells including BALB/c 3T3 fibroblast cells and smooth muscle cells, but unlike VEGFs, are not mitogenic for endothelial cells (Higashiyama et al., *Science* 251:936-939, 1991).

HBEGF also has a stimulatory effect on collateral vascularization and angiogenesis. Members of the HBEGF family are thought to play a pathophysiological role, for example, in a variety of tumors, such as bladder carcinomas, breast tumors and non-small cell lung tumors. Thus, these cell types are likely candidates for delivery of
5 cytocide-encoded agents.

HBEGF isolated from U-937 cells is heterogeneous in structure and contains at least 86 amino acids and two sites of *O*-linked glycosyl groups (Higashiyama et al., *J. Biol. Chem.* 267:6205-6212, 1992). The carboxyl-terminal half of the secreted HBEGF shares approximately 35% sequence identity with human EGF,
10 including six cysteines spaced in the pattern characteristic of members of the EGF protein family. In contrast, the amino-terminal portion of the mature factor is characterized by stretches of hydrophilic residues and has no structural equivalent in EGF. Site-directed mutagenesis of HBEGF and studies with peptide fragments have indicated that the heparin-binding sequences of HBEGF reside primarily in a 21 amino
15 acid stretch upstream of and slightly overlapping the EGF-like domain.

The effects of HBEGFs are mediated by EGF receptor tyrosine kinases expressed on cell surfaces of HBEGF-responsive cells (*see, e.g.*, U.S. Patent Nos. 5,183,884 and 5,218,090; and Ullrich et al., *Nature* 309:4113-425, 1984). The EGF receptor proteins, which are single chain polypeptides with molecular weights 170 kD,
20 constitute a family of structurally related EGF receptors. Cells known to express the EGF receptors include smooth muscle cells, fibroblasts, keratinocytes, and numerous human cancer cell lines, such as the: A431 (epidermoid); KB3-1 (epidermoid); COLO 205 (colon); CRL 1739 (gastric); HEP G2 (hepatoma); LNCAP (prostate); MCF-7 (breast); MDA-MB-468 (breast); NCI 417D (lung); MG63 (osteosarcoma); U-251
25 (glioblastoma); D-54MB (glioma); and SW-13 (adrenal).

For the purposes of this invention, HBEGF need only bind a specific HBEGF receptor and be internalized. Any member of the HBEGF family, whether or not it binds heparin, is useful within the context of this invention as long as it meets the requirements set forth above. Members of the HBEGF family are those that have
30 sufficient nucleotide identity to hybridize under normal stringency conditions (typically

greater than 75% nucleotide identity). Subfragments or subportions of a full-length HBEGF may also be desirable. One skilled in the art may find from the teachings provided within that certain biological activities are more or less desirable, depending upon the application.

5 DNA encoding an HBEGF peptide or polypeptide refers to any DNA fragment encoding an HBEGF, as defined above. Exemplary DNA fragments include: any such DNA fragments known to those of skill in the art; any DNA fragment that encodes an HBEGF or fragment that binds to an HBEGF receptor and is internalized thereby; and any DNA fragment that encodes any of the HBEGF polypeptides set forth
10 in SEQ ID NOs. 5-8. Such DNA sequences encoding HBEGF fragments are available from publicly accessible databases, such as: EMBL, GenBank (Accession Nos. M93012 (monkey) and M60278 (human)); the plasmid pMTN-HBEGF (ATCC #40900) and pAX-HBEGF (ATCC #40899) (described in PCT Application WO/92/06705); and Abraham et al., *Biochem. Biophys. Res. Comm.* 190:125-133, 1993). Unless modified
15 by replacement of degenerate codons, DNA encoding HBEGF polypeptides will hybridize under conditions of at least low stringency to DNA encoding a native human HBEGF (e.g., SEQ ID NO. 9). In addition, any DNA fragment that may be produced from any of the preceding DNA fragments by substitution of degenerate codons is also contemplated for use herein. It is understood that since the complete amino acid
20 sequence of HBEGF polypeptides, and DNA fragments encoding such peptides, are available to those of skill in this art, it is routine to substitute degenerate codons and produce any of the possible DNA fragments that encode such HBEGF polypeptides. It is also generally possible to synthesize DNA encoding such peptides based on the amino acid sequence.

25

4. Other receptor-binding internalized ligands

Other receptor-binding ligands may be used in the present invention. Any protein, polypeptide, analogue, or fragment that binds to a cell-surface receptor and is internalized may be used. In general, in addition to the specific heparin-binding
30 growth factors discussed above, other growth factors and cytokines are especially well suited for use. These ligands may be produced by recombinant or other means in

preparation for conjugation to the nucleic acid binding domain. The DNA sequences and methods to obtain the sequences of these receptor-binding internalized ligands are well known. For example, these ligands include CSF-1 (GenBank Accession Nos. M11038, M37435; Kawasaki et al., *Science* 230:291-296, 1985; Wong et al., *Science* 5 235:1504-1508, 1987); GM-CSF (GenBank Accession No. X03021; Miyatake et al., *EMBO J.* 4:2561-2568, 1985); IFN- α (interferon) (GenBank Accession No. A02076; Patent No. WO 8502862-A, July 4, 1985); IFN- γ (GenBank Accession No. A02137; Patent No. WO 8502624-A, June 20, 1985); hepatocyte growth factor (GenBank Accession No. X16323, S80567, X57574; Nakamura, et al., *Nature* 342:440-443, 1989; 10 Nakamura et al., *Prog. Growth Factor Res.* 3:67-85, 1991; Miyazawa et al., *Eur. J. Biochem.* 197:15-22, 1991); IGF-Ia (Insulin-like growth factor Ia) (GenBank Accession No. X56773, S61841; Sandberg-Nordqvist et al., *Brain Res. Mol. Brain Res.* 12:275-277, 1992; Sandberg, Sandberg-Nordqvist et al., *Cancer Res.* 53:2475-2478, 1993); IGF-Ib (GenBank Accession No. X56774 S61860; Sandberg-Nordqvist et al., *Brain* 15 *Res. Mol. Brain Res.* 12:275-277, 1992; Sandberg-Nordqvist, A.C., *Cancer Res.* 53:2475-2478, 1993); IGF-I (GenBank Accession No. X03563, M29644; Dull et al., *Nature* 310:771-781, 1984; Rall et al., *Meth. Enzymol.* 146:239-248, 1987); IGF-II (GenBank Accession No. J03242; Shen et al., *Proc. Natl. Acad. Sci. USA* 85:1947-1951, 1988); IL-1- α (interleukin 1 alpha) (GenBank Accession No. X02531, M15329; 20 March et al., *Nature* 315:641-647, 1985; Nishida et al., *Biochem. Biophys. Res. Commun.* 143:345-352, 1987); IL-1- β (interleukin 1 beta) (GenBank Accession No. X02532, M15330, M15840; March et al., *Nature* 315:641-647, 1985; Nishida et al., *Biochem. Biophys. Res. Commun.* 143:345-352, 1987; Bensi et al., *Gene* 52:95-101, 1987); IL-1 (GenBank Accession No. K02770, M54933, M38756; Auron et al., *Proc.* 25 *Natl. Acad. Sci. USA* 81:7907-7911, 1984; Webb et al., *Adv. Gene Technol.* 22:339-340, 1985); IL-2 (GenBank Accession No. A14844, A21785, X00695, X00200, X00201, X00202; Lupker et al., Patent No. EP 0307285-A, March 15, 1989; Perez et al., Patent No. EP 0416673-A, March 13, 1991; Holbrook et al., *Nucleic Acids Res.* 12:5005-5013, 1984; Degrove et al., *EMBO J.* 2:2349-2353, 1983; Taniguchi et al., *Nature* 302:305-310, 1983); IL-3 (GenBank Accession No. M14743, M20137; Yang et al., *Cell* 47:3-10, 30

1986; Otsuka et al., *J. Immunol.* 140:2288-2295, 1988); IL-4 (GenBank Accession No. M13982; Yokota et al., *Proc. Natl. Acad. Sci. USA* 83:5894-5898, 1986); IL-5 (GenBank Accession No. X04688, J03478; Azuma et al., *Nucleic Acids Res.* 14:9149-9158, 1986; Tanabe et al., *J. Biol. Chem.* 262:16580-16584, 1987); IL-6 (GenBank
5 Accession No. Y00081, X04602, M54894, M38669, M14584; Yasukawa et al., *EMBO J.* 6:2939-2945, 1987; Hirano et al., *Nature* 324:73-76, 1986; Wong et al., *Behring Inst. Mitt.* 83:40-47, 1988; May et al., *Proc. Natl. Acad. Sci. USA* 83:8957-8961, 1986); IL-7 (GenBank Accession No. J04156; Goodwin et al., *Proc. Natl. Acad. Sci. USA* 86:302-306, 1989); IL-8 (GenBank Accession No. Z11686; Kusner et al., *Kidney Int.* 39:1240-
10 1248, 1991); IL-10 (GenBank Accession No. X78437, M57627; Vieira et al., *Proc. Natl. Acad. Sci. USA* 88:1172-1176, 1991); IL-11 (GenBank Accession No. M57765 M37006; Paul et al., *Proc. Natl. Acad. Sci. USA* 87:7512-7516, 1990); IL-13 (GenBank Accession No. X69079, U10307; Minty et al., *Nature* 362:248-250, 1993; Smirnov, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, June 2, 1994); TNF- α
15 (Tumor necrosis factor) (GenBank Accession No. A21522; Patent No. GB 2246569-A1, February 5, 1992); TNF- β (GenBank Accession No. D12614; Matsuyama et al., *FEBS LETTERS* 302:141-144, 1992). DNA sequences of other suitable receptor-binding internalized ligands may be obtained from GenBank or EMBL DNA databases, reverse-synthesized from protein sequence obtained from PIR database or isolated by standard
20 methods (Sambrook et al., *supra*) from cDNA or genomic libraries.

5. Modifications of receptor-binding internalized ligands

These ligands may be customized for a particular application. Means for modifying proteins is provided below. Briefly, additions, substitutions and deletions of
25 amino acids may be produced by any commonly employed recombinant DNA method.

An amino acid residue of FGF, VEGF, HBEGF or other receptor-binding internalized ligand is non-essential if the polypeptide that has been modified by deletion of the residue possesses substantially the same ability to bind to its receptor and internalize a linked agent as the unmodified polypeptide.

30 As noted above, any polypeptide or peptide analogue, including peptidomimetics, that is reactive with an FGF receptor, a VEGF receptor, an HBEGF

receptor, other growth factor receptor (*e.g.*, PDGF receptor), cytokine receptor or other cell surface molecule including members of the families and fragments thereof, or constrained analogs of such peptides that bind to the receptor and internalize a linked targeted agent may be used in the context of this invention. Members of the FGF
5 peptide family, including FGF-1 to FGF-9, are preferred. Modified peptides, especially those lacking proliferative function, and chimeric peptides, which retain the specific binding and internalizing activities are also contemplated for use herein.

A modification that is effected substantially near the N-terminus of a polypeptide is generally effected within the first about ten residues of the protein. Such
10 modifications include the addition or deletion of residues, such as the addition of a cysteine to facilitate conjugation and form conjugates that contain a defined molar ratio, preferably a ratio of 1:1 of the polypeptides.

DNA encoding one of the receptor-binding internalized ligands discussed above may be mutagenized using standard methodologies to delete or replace
15 any cysteine residues that are responsible for aggregate formation. If necessary, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or replacing a cysteine residue and ascertaining whether the resulting protein aggregates in solutions containing physiologically acceptable buffers and salts. In addition, fragments of these receptor-binding internalized ligands may be
20 constructed and used. The binding region of many of these ligands have been delineated. Fragments may also be shown to bind and internalize by any one of the tests described herein.

Modification of the polypeptide may be effected by any means known to those of skill in this art. The preferred methods herein rely on modification of DNA
25 encoding the polypeptide and expression of the modified DNA.

Merely by way of example, DNA encoding the FGF polypeptide may be isolated, synthesized or obtained from commercial sources (the amino acid sequences of FGF-1 - FGF-9 are set forth in SEQ ID NOs. 10-18; DNA sequences may be based on these amino acid sequences or may be obtained from public DNA databases and
30 references (*see, e.g.*, GenBank, *see also* U.S. Patent No. 4,956,455, U.S. Patent

No. 5,126,323, U.S. Patent No. 5,155,217, U.S. Patent No. 4,868,113, PCT Application WO 90/08771, EP Application 0 488 196 A2, and Miyamoto et al., *Mol. Cell. Biol.* 13:4251-4259, 1993). Expression of a recombinant FGF-2 protein in yeast and *E. coli* is described in Barr et al., *J. Biol. Chem.* 263:16471-16478, 1988, in PCT Application
5 Serial No. PCT/US93/05702 and United States Application Serial No. 07/901,718. Expression of recombinant FGF proteins may be performed as described herein or using methods known to those of skill in the art.

Similarly, DNA encoding any of the other receptor-binding internalized ligands, including VEGF, HBEGF, IL-1, IL-2, and other cytokines and growth factors
10 may also be isolated, synthesized, or obtained from commercial sources. As noted above, DNA sequences are available in public databases, such as GenBank. Based on these sequences, oligonucleotide primers may be designed and used to amplify the gene from cDNA or mRNA by polymerase chain reaction technique as one means of obtaining DNA.

15 Mutations may be made by any method known to those of skill in the art, including site-specific or site-directed mutagenesis of DNA encoding the protein and the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and
20 double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used (*see, e.g., Veira et al., Meth. Enzymol.* 15:3, 1987). In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (*i.e., a member of the FGF family or a cytotoxic*
25 *molecule, such as a saporin*). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other
30 the original sequence. The heteroduplex is introduced into appropriate bacterial cells

and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

Suitable conservative substitutions of amino acids are well-known and may be made generally without altering the biological activity of the resulting molecule. For example, such substitutions may be made in accordance with those set forth in TABLE 1 as follows:

TABLE 1

10

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Other similarly conservative substitutions may be made. If necessary, such substitutions may be determined empirically merely by testing the resulting modified protein for the ability to bind to and internalize upon binding to the appropriate

receptors. Those that retain this ability are suitable for use in the conjugates and methods herein. In addition, muteins of the FGFs are known to those of skill in the art (see, e.g., U.S. Patent No. 5,175,147; PCT Application No. WO 89/00198, U.S. Serial No. 07/070,797; PCT Application No. WO 91/15229; and U.S. Serial No. 07/505,124).

5

B. Nucleic acid binding domains

As previously noted, nucleic acid binding domains (NABD) interact with the target nucleic acid either in a sequence-specific manner or a sequence-nonspecific manner. When the interaction is non-specific, the nucleic acid binding domain binds
10 nucleic acid regardless of the sequence. For example, poly-L-lysine is a basic polypeptide that binds to oppositely charged DNA. Other highly basic proteins or polycationic compounds, such as histones, protamines, and spermidine, also bind to nucleic acids in a nonspecific manner.

Many proteins have been identified that bind specific sequences of DNA.
15 These proteins are responsible for genome replication, transcription and repair of damaged DNA. The transcription factors regulate gene expression and are a diverse group of proteins. These factors are especially well suited for purposes of the subject invention because of their sequence-specific recognition. Host transcription factors have been grouped into seven well-established classes based upon the structural motif
20 used for recognition. The major families include helix-turn-helix (HTH) proteins, homeodomains, zinc finger proteins, steroid receptors, leucine zipper proteins, the helix-loop-helix (HLH) proteins, and β -sheets. Other classes or subclasses may eventually be delineated as more factors are discovered and defined. Proteins from those classes or proteins that do not fit within one of these classes but bind nucleic acid
25 in a sequence-specific manner, such as SV40 T antigen and p53 may also be used.

These families of transcription factors are generally well-known (see GenBank; Pabo and Sauer, *Ann. Rev. Biochem.* 61:1053-1095, 1992; and references below). Many of these factors are cloned and the precise DNA-binding region delineated in certain instances. When the sequence of the DNA-binding domain is
30 known, a gene encoding it may be synthesized if the region is short. Alternatively, the genes may be cloned from the host genomic libraries or from cDNA libraries using

oligonucleotides as probes or from genomic DNA or cDNA by polymerase chain reaction methods. Such methods may be found in Sambrook et al., *supra*.

Helix-turn-helix proteins include the well studied λ Cro protein, λ CI, and *E. coli* CAP proteins (see Steitz et al., *Proc. Natl. Acad. Sci. USA* 79:3097-3100, 1982; 5 Ohlendorf et al., *J. Mol. Biol.* 169:757-769, 1983). In addition, the *lac* repressor (Kaptein et al., *J. Mol. Biol.* 182:179-182, 1985) and Trp repressor (Scheritz et al., *Nature* 317:782-786, 1985) belong to this family. Members of the homeodomain family include the *Drosophila* protein Antennapedia (Qian et al., *Cell* 59:573-580, 1989) and yeast MAT α 2 (Wolberger et al., *Cell* 67:517-528, 1991). Zinc finger 10 proteins include TFIIIA (Miller et al., *EMBO J.* 4:1609-1614, 1985), Sp-1, zif 268, and many others (see generally Krizek et al., *J. Am. Chem. Soc.* 113:4518-4523, 1991). Steroid receptor proteins include receptors for steroid hormones, retinoids, vitamin D, thyroid hormones, as well as other compounds. Specific examples include retinoic acid, knirps, progesterone, androgen, glucocorticoid and estrogen receptor proteins. The 15 leucine zipper family was defined by a heptad repeat of leucines over a region of 30 to 40 residues. Specific members of this family include C/EBP, *c-fos*, *c-jun*, GCN4, sis-A, and CREB (see generally O'Shea et al., *Science* 254:539-544, 1991). The helix-loop-helix (HLH) family of proteins appears to have some similarities to the leucine zipper family. Well-known members of this family include myoD (Weintraub et al., *Science* 20 251:761-766, 1991); *c-myc*; and AP-2 (Williams and Tijan, *Science* 251:1067-1071, 1991). The β -sheet family uses an antiparallel β -sheet for DNA binding, rather than the more common α -helix. The family contains the MetJ (Phillips, *Curr. Opin. Struc. Biol.* 1:89-98, 1991), Arc (Breg et al., *Nature* 346:586-589, 1990) and Mnt repressors. In addition, other motifs are used for DNA binding, such as the cysteine-rich motif in yeast 25 GAL4 repressor, and the GATA factor. Viruses also contain gene products that bind specific sequences. One of the most-studied such viral genes is the *rev* gene from HIV. The *rev* gene product binds a sequence called RRE (*rev* responsive element) found in the *env* gene. Other proteins or peptides that bind DNA may be discovered on the basis of sequence similarity to the known classes or functionally by selection.

Several techniques may be used to select other nucleic acid binding domains (see U.S. Patent No. 5,270,170; PCT Application WO 93/14108; and U.S. Patent No. 5,223,409). One of these techniques is phage display. (See, for example, U.S. Patent No. 5,223,409.) In this method, DNA sequences are inserted into the

5 gene III or gene VIII gene of a filamentous phage, such as M13. Several vectors with multicloning sites have been developed for insertion (McLafferty et al., *Gene* 128:29-36, 1993; Scott and Smith, *Science* 249:386-390, 1990; Smith and Scott, *Methods Enzymol.* 217:228-257, 1993). The inserted DNA sequences may be randomly generated or variants of a known DNA-binding domain. Generally, the inserts encode

10 from 6 to 20 amino acids. The peptide encoded by the inserted sequence is displayed on the surface of the bacteriophage. Bacteriophage expressing a desired nucleic acid-binding domain are selected for by binding to the cytocide-encoding agent. This target molecule may be single stranded or double stranded DNA or RNA. When the cytocide-encoding agent to be delivered is single-stranded, such as RNA, the appropriate target is

15 single-stranded. When the molecule to be delivered is double-stranded, the target molecule is preferably double-stranded. Preferably, the entire coding region of the cytocide-encoding agent is used as the target. In addition, elements necessary for transcription that are included for *in vivo* or *in vitro* delivery may be present in the target DNA molecule. Bacteriophage that bind the target are recovered and propagated.

20 Subsequent rounds of selection may be performed. The final selected bacteriophage are propagated and the DNA sequence of the insert is determined. Once the predicted amino acid sequence of the binding peptide is known, sufficient peptide for use herein as an nucleic acid binding domain may be made either by recombinant means or synthetically. Recombinant means is used when the receptor-binding internalized

25 ligand/nucleic acid binding domain is produced as a fusion protein. In addition, the peptide may be generated as a tandem array of two or more peptides, in order to maximize affinity or binding of multiple DNA molecules to a single polypeptide.

As an example of the phage display selection technique, a DNA-binding domain/peptide that recognizes the coding region of saporin is isolated. Briefly, DNA

30 fragments encoding saporin may be isolated from a plasmid containing these sequences.

The plasmid FPFS1 contains the entire coding region of saporin. Digestion of the plasmid with *NcoI* and *EcoRI* restriction enzymes liberates the saporin specific sequence as a single fragment of approximately 780 bp. This fragment may be purified by any one of a number of methods, such as agarose gel electrophoresis and subsequent
5 elution from the gel. The saporin fragment is fixed to a solid support, such as in the wells of a 96-well plate. If the double-stranded fragment does not bind well to the plate, a coating such as a positively charged molecule, may be used to promote DNA adherence. The phage library is added to the wells and an incubation period allows for binding of the phage to the DNA. Unbound phage are removed by a wash, typically
10 containing 10 mM Tris, 1 mM EDTA, and without salt or with a low salt concentration. Bound phage are eluted starting at a 0.1 M NaCl containing buffer. The NaCl concentration is increased in a step-wise fashion until all the phage are eluted. Typically, phage binding with higher affinity will only be released by higher salt concentrations.

15 Eluted phage are propagated in the bacteria host. Further rounds of selection may be performed to select for a few phage binding with high affinity. The DNA sequence of the insert in the binding phage is then determined. In addition, peptides having a higher affinity may be isolated by making variants of the insert sequence and subjecting these variants to further rounds of selection.

20

C. Cytocide-encoding agents

A cytocide-encoding agent is a nucleic acid molecule (DNA or RNA) that, upon internalization by a cell, and subsequent transcription (if DNA) and[/or] translation into a cytotoxic agent, is cytotoxic to a cell or inhibits cell growth by
25 inhibiting protein synthesis.

Cytocides include saporin, the ricins, abrin and other ribosome inactivating proteins, *Pseudomonas* exotoxin, diphtheria toxin, angiogenin, tritin, dianthins 32 and 30, momordin, pokeweed antiviral protein, mirabilis antiviral protein, bryodin, angiogenin, and shiga exotoxin, as well as other cytocides that are known to
30 those of skill in the art. Alternatively, cytocide gene products may be noncytotoxic but

activate a compound, which is endogenously produced or exogenously applied, from a nontoxic form to a toxic product that inhibits protein synthesis.

Especially of interest are DNA molecules that encode an enzyme that results in cell death or renders a cell susceptible to cell death upon the addition of another product. For example, saporin is an enzyme that cleaves rRNA and inhibits protein synthesis. Other enzymes that inhibit protein synthesis are especially well suited for use in the present invention. In addition, enzymes may be used where the enzyme activates a compound with little or no cytotoxicity into a toxic product that inhibits protein synthesis.

10

1. Ribosome inactivating proteins

Ribosome-inactivating proteins (RIPs), which include ricin, abrin, and saporin, are plant proteins that catalytically inactivate eukaryotic ribosomes. Ribosome-inactivating proteins inactivate ribosomes by interfering with the protein elongation step of protein synthesis. For example, the ribosome-inactivating protein saporin (hereinafter also referred to as SAP) has been shown to inactivate 60S ribosomes by cleavage of the N-glycosidic bond of the adenine at position 4324 in the rat 28S ribosomal RNA (rRNA). The particular region in which A₄₃₂₄ is located in the rRNA is highly conserved among prokaryotes and eukaryotes; A₄₃₂₄ in 28S rRNA corresponds to A₂₆₆₀ in *E. coli* 23S rRNA. Several of the ribosome inactivating proteins also appear to interfere with protein synthesis in prokaryotes, such as *E. coli*.

Saporin is preferred as a cytocide, but other suitable ribosome inactivating proteins (RIPs) and toxins may be used. Other suitable RIPs include, but are not limited to, ricin, ricin A chain, maize ribosome inactivating protein, gelonin, diphtheria toxin, diphtheria toxin A chain, trichosanthin, tritin, pokeweed antiviral protein (PAP), mirabilis antiviral protein (MAP), Dianthins 32 and 30, abrin, monordin, bryodin, shiga (see, e.g., WO 93/24620) and others (see, e.g., Barbieri et al., *Cancer Surveys* 1:489-520, 1982, and European patent application No. 0466 222, incorporated herein by reference, which provide lists of numerous ribosome inactivating proteins and their sources; see also U.S. Patent No. 5,248,608 to Walsh et al.). Some ribosome inactivating proteins, such as abrin and ricin, contain two constituent chains: a cell-

binding chain that mediates binding to cell surface receptors and internalization of the molecule and a chain responsible for toxicity. Single chain ribosome inactivating proteins (type I RIPS), such as the saporins, do not have a cell-binding chain. As a result, unless internalized, they are substantially less toxic to whole cells than the
5 ribosome inactivating proteins that have two chains.

Several structurally related ribosome inactivating proteins have been isolated from seeds and leaves of the plant *Saponaria officinalis* (soapwort) (GB Patent 2,194,241 B; GP Patent 2,216,891; EP Patent 89306016). Saporin proteins for use in this invention have amino acid sequences found in the natural plant host *Saponaria*
10 *officinalis* or modified sequences, having amino acid substitutions, deletions, insertions or additions, but which still express substantial ribosome inactivating activity. Purified preparations of saporin are frequently observed to include several molecular isoforms of the protein. It is understood that differences in amino acid sequences can occur in saporin from different species as well as between saporin molecules from individual
15 organisms of the same species. Among these, SO-6 is the most active and abundant, representing 7% of total seed proteins. Saporin is very stable, has a high isoelectric point, does not contain carbohydrates, and is resistant to denaturing agents, such as sodium dodecyl sulfate (SDS), and a variety of proteases. The amino acid sequences of several saporin-6 isoforms from seeds are known, and there appear to be families of
20 saporin ribosome inactivating proteins differing in few amino acid residues. Any of these saporin proteins or modified proteins that are cytotoxic may be used in the present invention.

a. Isolation of DNA encoding saporin

25 Some of the DNA molecules provided herein encode saporin that has substantially the same amino acid sequence and ribosome inactivating activity as that of saporin-6 (SO-6), including any of four isoforms, which have heterogeneity at amino acid positions 48 and 91 (see, e.g., Maras et al., *Biochem. Internat.* 21:631-638, 1990, and Barra et al., *Biotechnol. Appl. Biochem.* 13:48-53, 1991; GB Patent 2,216,891 B
30 and EP Patent 89306106; and SEQ ID NOs. 19-23). Other suitable saporin polypeptides include other members of the multi-gene family coding for isoforms of

5 saporin-type ribosome inactivating proteins including SO-1 and SO-3 (Fordham-Skelton et al., *Mol. Gen. Genet.* 221:134-138, 1990), SO-2 (*see, e.g.*, U.S. Application Serial No. 07/885,242; GB 2,216,891; *see also* Fordham-Skelton et al., *Mol. Gen. Genet.* 229:460-466, 1991), SO-4 (*see, e.g.*, GB 2,194,241 B; *see also* Lappi et al.,
10 *Biochem. Biophys. Res. Commun.* 129:934-942, 1985) and SO-5 (*see, e.g.*, GB 2,194,241 B; *see also* Montecucchi et al., *Int. J. Peptide Protein Res.* 33:263-267, 1989).

The saporin polypeptides for use in this invention include any of the isoforms of saporin that may be isolated from *Saponaria officinalis* or related species or
10 modified forms that retain cytotoxic activity. In particular, such modified saporin may be produced by modifying the DNA encoding the protein (*see, e.g.*, International PCT Application Serial No. PCT/US93/05702, and United States Application Serial No. 07/901,718; *see also* U.S. Patent Application No. 07/885,242, and Italian Patent No. 1,231,914) by altering one or more amino acids or deleting or inserting one or more
15 amino acids. Any such protein, or portion thereof, that exhibits cytotoxicity in standard *in vitro* or *in vivo* assays within at least about an order of magnitude of the saporin conjugates described herein is contemplated for use herein.

Preferably, the saporin DNA sequence contains mammalian-preferred codons (SEQ. ID NO. 79). Preferred codon usage as exemplified in *Current Protocols*
20 *in Molecular Biology, infra*, and Zhang et al. (*Gene* 105:61, 1991) for mammals, yeast, *Drosophila*, *E. coli*, and primates is established for saporin sequence.

The cytocide-encoding agent, such as saporin DNA sequence, is introduced into a plasmid in operative linkage to an appropriate promoter for expression of polypeptides in the organism. The presently preferred saporin proteins are SO-6 and
25 SO-4. The DNA can optionally include sequences, such as origins of replication that allow for the extrachromosomal maintenance of the saporin-containing plasmid, or can be designed to integrate into the genome of the host (as an alternative means to ensure stable maintenance in the host).

b. Nucleic acids encoding other ribosome inactivating proteins and cytocides

In addition to saporin discussed above, other cytocides that inhibit protein synthesis are useful in the present invention. The gene sequences for these
5 cytocides may be isolated by standard methods, such as PCR, probe hybridization of genomic or cDNA libraries, antibody screenings of expression libraries, or clones may be obtained from commercial or other sources. The DNA sequences of many of these cytocides are well known, including ricin A chain (GenBank Accession No. X02388); maize ribosome inactivating protein (GenBank Accession No. L26305); gelonin
10 (GenBank Accession No. L12243; PCT Application WO 92/03155; U.S. Patent No. 5,376,546; diphtheria toxin (GenBank Accession No. K01722); trichosanthin (GenBank Accession No. M34858); tritin (GenBank Accession No. D13795); pokeweed antiviral protein (GenBank Accession No. X78628); mirabilis antiviral protein (GenBank Accession No. D90347); dianthin 30 (GenBank Accession
15 No. X59260); abrin (GenBank Accession No. X55667); shiga (GenBank Accession No. M19437) and *Pseudomonas* exotoxin (GenBank Accession Nos. K01397, M23348). When DNA sequences or amino acid sequences are known, DNA molecules encoding these proteins may be synthesized, and preferably contain mammalian-preferred codons.

20

D. Prodrug-encoding agent

A nucleic acid molecule encoding a prodrug may alternatively be used within the context of the present invention. Prodrugs are inactive in the host cell until either a substrate is provided or an activating molecule is provided. Most typically, a
25 prodrug activates a compound with little or no cytotoxicity into a toxic product. Two of the more often used prodrug molecules, both of which may be used in the present invention, are HSV thymidine kinase and *E. coli* cytosine deaminase.

Briefly, a wide variety of gene products which either directly or indirectly activate a compound with little or no cytotoxicity into a toxic product may be
30 utilized within the context of the present invention. Representative examples of such gene products include HSVTK (herpes simplex virus thymidine kinase) and VZVTK

(varicella zoster virus thymidine kinase), which selectively phosphorylate certain purine arabinosides and substituted pyrimidine compounds. Phosphorylation converts these compounds to metabolites that are cytotoxic or cytostatic. For example, exposure of the drugs ganciclovir, acyclovir, or any of their analogues (*e.g.*, FIAU, FIAC, DHPG) to
5 cells expressing HSVTK allows conversion of the drug into its corresponding active nucleotide triphosphate form.

Other gene products that may be utilized within the context of the present invention include *E. coli* guanine phosphoribosyl transferase, which converts thioxanthine into toxic thioxanthine monophosphate (Besnard et al., *Mol. Cell. Biol.*
10 7:4139-4141, 1987); alkaline phosphatase, which converts inactive phosphorylated compounds such as mitomycin phosphate and doxorubicin-phosphate to toxic dephosphorylated compounds; fungal (*e.g.*, *Fusarium oxysporum*) or bacterial cytosine deaminase, which converts 5-fluorocytosine to the toxic compound 5-fluorouracil (Mullen, *PNAS* 89:33, 1992); carboxypeptidase G2, which cleaves glutamic acid from
15 para-N-bis (2-chloroethyl) aminobenzoyl glutamic acid, thereby creating a toxic benzoic acid mustard; and Penicillin-V amidase, which converts phenoxyacetabide derivatives of doxorubicin and melphalan to toxic compounds (see generally, Vrudhula et al., *J. of Med. Chem.* 36(7):919-923, 1993; Kern et al., *Canc. Immun. Immunother.* 31(4):202-206, 1990). Moreover, a wide variety of *Herpesviridae* thymidine kinases,
20 including both primate and non-primate herpesviruses, are suitable. Such herpesviruses include Herpes Simplex Virus Type 1 (McKnight et al., *Nuc. Acids Res* 8:5949-5964, 1980), Herpes Simplex Virus Type 2 (Swain and Galloway, *J. Virol.* 46:1045-1050, 1983), Varicella Zoster Virus (Davison and Scott, *J. Gen. Virol.* 67:1759-1816, 1986), marmoset herpesvirus (Otsuka and Kit, *Virology* 135:316-330, 1984), feline herpesvirus
25 type 1 (Nunberg et al., *J. Virol.* 63:3240-3249, 1989), pseudorabies virus (Kit and Kit, U.S. Patent No. 4,514,497, 1985), equine herpesvirus type 1 (Robertson and Whalley, *Nuc. Acids Res.* 16:11303-11317, 1988), bovine herpesvirus type 1 (Mittal and Field, *J. Virol* 70:2901-2918, 1989), turkey herpesvirus (Martin et al., *J. Virol.* 63:2847-2852, 1989), Marek's disease virus (Scott et al., *J. Gen. Virol.* 70:3055-3065, 1989),
30 herpesvirus saimiri (Honess et al., *J. Gen. Virol.* 70:3003-3013, 1989) and Epstein-Barr

virus (Baer et al., *Nature (London)* 310:207-311, 1984). Such herpesviruses may be readily obtained from commercial sources such as the American Type Culture Collection ("ATCC", Rockville, Maryland).

Furthermore, as indicated above, a wide variety of inactive precursors
5 may be converted into active inhibitors. For example, thymidine kinase can phosphorylate nucleosides (*e.g.*, dT) and nucleoside analogues such as ganciclovir (9-
{{[2-hydroxy-1-(hydroxymethyl)ethoxyl methyl} guanosine), famciclovir, buciclovir,
penciclovir, valciclovir, acyclovir (9-[2-hydroxy ethoxy)methyl] guanosine),
trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A
10 (adenosine arabinoside, vivarabine), 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-
deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine (5-iodo-2'-
deoxyuridine), AZT (3' azido-3' thymidine), ddC (dideoxycytidine), AIU (5-iodo-5'
amino 2', 5'-dideoxyuridine) and AraC (cytidine arabinoside).

15 E. Other nucleic acid molecules

The conjugates provided herein may also be used to deliver other types
of nucleic acids to targeted cells. Such other nucleic acids include antisense RNA,
antisense DNA, ribozymes, triplex-forming oligonucleotides, and oligonucleotides that
bind proteins. The nucleic acids can also include RNA trafficking signals, such as viral
20 packaging sequences (*see, e.g.*, Sullenger et al. (1994) *Science* 262:1566-1569). The
nucleic acids also include DNA molecules that encode proteins that replace defective
genes, such as the gene associated with cystic fibrosis (*see, e.g.*, PCT Application WO
93/03709, U.S. Application Serial No. 07/745,900; and Riordan et al. (1989) *Science*
245:1066-1073). Other DNA molecules may encode tumor-specific cytotoxic
25 molecules, such as tumor necrosis factor, viral antigens and other proteins to render a
cell susceptible to anti-cancer agents.

Nucleic acids and oligonucleotides for use as described herein can be
synthesized by any method known to those of skill in this art (*see, e.g.*, WO 93/01286,
U.S. Application Serial No. 07/723,454; U.S. Patent No. 5,218,088; U.S. Patent No.
30 5,175,269; U.S. Patent No. 5,109,124). Identification of oligonucleotides and
ribozymes for use as antisense agents and DNA encoding genes for targeted delivery for

genetic therapy involve methods well known in the art. For example, the desirable properties, lengths and other characteristics of such oligonucleotides are well known. Antisense oligonucleotides are typically designed to resist degradation by endogenous nucleolytic enzymes and include, but are not limited to: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (*see, e.g., Agrwal et al., Tetrahedron Lett.* 28:3539-3542 (1987); Miller et al., *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stec et al., *Tetrahedron Lett.* 26:2191-2194 (1985); Moody et al., *Nucl. Acids Res.* 12:4769-4782 (1989); Uznanski et al., *Nucl. Acids Res.* (1989); Letsinger et al., *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367-402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry* 27:7237-7246 (1988)).

Antisense nucleotides are oligonucleotides that bind in a sequence-specific manner to nucleic acids, such as mRNA or DNA. When bound to mRNA that has complementary sequences, antisense prevents translation of the mRNA (*see, e.g., U.S. Patent No. 5,168,053 to Altman et al.; U.S. Patent No. 5,190,931 to Inouye, U.S. Patent No. 5,135,917 to Burch; U.S. Patent No. 5,087,617 to Smith and Clusel et al. (1993) Nucl. Acids Res.* 21:3405-3411, which describes dumbbell antisense oligonucleotides). Triplex molecules refer to single DNA strands that bind duplex DNA forming a colinear triplex molecule and thereby prevent transcription (*see, e.g., U.S. Patent No. 5,176,996 to Hogan et al., which describes methods for making synthetic oligonucleotides that bind to target sites on duplex DNA*).

Particularly useful antisense nucleotides and triplex molecules are molecules that are complementary or bind to the sense strand of DNA or mRNA that encodes an oncogene, such as bFGF, int-2, hst-1/K-FGF, FGF-5, hst-2/FGF-6, FGF-8. Other useful antisense oligonucleotides include those that are specific for IL-8 (*see, e.g., U.S. Patent No. 5,241,049; and PCT Applications WO 89/004836; WO 90/06321; WO 89/10962; WO 90/00563; and WO 91/08483*), which can be linked to bFGF for the treatment of psoriasis, anti-sense oligonucleotides that are specific for nonmuscle

myosin heavy chain and/or *c-myb* (see, e.g., Simons et al. (1992) *Circ. Res.* 70:835-843; PCT Application WO 93/01286, U.S. application Serial No. 07/723,454; LeClerc et al. (1991) *J. Am. Coll. Cardiol.* 17 (2 Suppl. A):105A; Ebbecke et al. (1992) *Basic Res. Cardiol.* 87:585-591), which can be targeted by an FGF to inhibit smooth muscle cell proliferation, such as that following angioplasty and thereby prevent restenosis or inhibit viral gene expression in transformed or infected cells.

A ribozyme is an RNA molecule that specifically cleaves RNA substrates, such mRNA, and thus inhibits or interferes with cell growth or expression. There are at least five classes of ribozymes that are known that are involved in the cleavage and/or ligation of RNA chains. Ribozymes can be targeted to any RNA transcript and can catalytically cleave such transcript (see, e.g., U.S. Patent No. 5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246 to Cech et al., which described ribozymes and methods for production thereof). Any such ribosome may be linked to the growth factor for delivery to a cell bearing a receptor for a receptor-internalized binding ligand.

The ribozymes may be delivered to the targeted cells by DNA encoding the ribozyme linked to a eukaryotic promoter, such as a eukaryotic viral promoter, such that upon introduction into the nucleus, the ribozyme will be directly transcribed. In such instances, the construct will also include a nuclear translocation sequence, generally as part of the ligand or as part of a linker between the ligand and nucleic acid binding domain.

DNA that encodes a therapeutic product contemplated for use includes DNA encoding correct copies of defective genes, such as the defective gene (CFTR) associated with cystic fibrosis (see, e.g., International Application WO 93/03709, U.S. Application Serial No. 07/745,900; and Riordan et al. (1989) *Science* 245:1066-1073), and anticancer agents, such as tumor necrosis factors. The conjugate preferably includes an NTS. If the conjugate is designed such that the ligand and nucleic acid binding domain are cleaved in the cytoplasm, then the NTS should be included in a portion of the conjugate or linker that remains bound to the DNA. The nuclear

translocation sequence (NTS) may be a heterologous sequence or a may be derived from the selected growth factor.

F. Construct containing cytotoxic-encoding agent

5 In the case of cytotoxic molecules such as the ribosome inactivating proteins, very few molecules may need to be expressed to effect cell killing. Indeed, only a single molecule of diphtheria toxoid introduced into a cell was sufficient to kill the cell. With other cytotoxins or prodrugs, it may be that propagation or stable maintenance of the construct is necessary to attain a sufficient amount or concentration
10 of the gene product for effective gene therapy. Examples of replicating and stable eukaryotic plasmids may be found in the scientific literature.

In general, constructs will also contain elements necessary for transcription and translation. If the cytotoxic-encoding agent is DNA, then it must contain a promoter. The choice of the promoter will depend upon the cell type to be
15 transformed and the degree or type of control desired. Promoters can be constitutive or active in any cell type, tissue specific, cell specific, event specific temporal-specific or inducible. Cell-type specific promoters and event type specific promoters are preferred. Examples of constitutive or nonspecific promoters include the SV40 early promoter (U.S. Patent No. 5,118,627), the SV40 late promoter (U.S. Patent No. 5,118,627), CMV
20 early gene promoter (U.S. Patent No. 5,168,062), and adenovirus promoter. In addition to viral promoters, cellular promoters are also amenable within the context of this invention. In particular, cellular promoters for the so-called housekeeping genes are useful. Viral promoters are preferred, because generally they are stronger promoters than cellular promoters.

25 Tissue specific promoters are particularly useful when a certain tissue type is to be targeted for transformation. By using one of this class of promoters, an extra margin of specificity can be attained. For example, when the indication to be treated is ophthalmological (*e.g.*, secondary lens clouding), either the alpha-crystalline promoter or gamma-crystalline promoter is preferred. When a tumor is the target of
30 gene delivery, cellular promoters for specific tumor markers or promoters more active in tumor cells should be chosen. Thus, to treat prostate tumor, the prostate-specific

antigen promoter is especially useful. Similarly, the tyrosinase promoter or tyrosinase-related protein promoter is a preferred promoter for melanoma treatment. For treatment of diseases that are angiogenic or exacerbated by angiogenesis, the VEGF receptor promoter is preferred. The VEGF receptor is expressed in developing capillaries. For
5 treatment of breast cancer, the promoter from heat shock protein 27 is preferred; for treatment of colon or lung cancer, the promoter from carcinoembryonic antigen is preferred; for treatment of restenosis or other diseases involving smooth muscle cells, the promoter from α -actin or myosin heavy chain is preferred. For B lymphocytes, the immunoglobulin variable region gene promoter; for T lymphocytes, the TCR receptor
10 variable region promoter; for helper T lymphocytes, the CD4 promoter; for liver, the albumin or α -fetoprotein promoter, are a few additional examples of tissue specific promoters. Many other examples of tissue specific promoters are readily available to one skilled in the art. Some of these promoters are temporally regulated, such as *c-myc* and cyclin D.

15 Inducible promoters may also be used. These promoters include the MMTV LTR (PCT WO 91/13160), which is inducible by dexamethasone, metallothionein, which is inducible by heavy metals, and promoters with cAMP response elements, which are inducible by cAMP. By using an inducible promoter, the nucleic acid may be delivered to a cell and will remain quiescent until the addition of
20 the inducer. This allows further control on the timing of production of the therapeutic gene.

Event-type specific promoters are active or up-regulated only upon the occurrence of an event, such as tumorigenicity or viral infection. The HIV LTR is a well known example of an event-specific promoter. The promoter is inactive unless the
25 *tat* gene product is present, which occurs upon viral infection. Another promoter is *c-myc*.

Additionally, promoters that are coordinately regulated with a particular cellular gene may be used. For example, promoters of genes that are coordinately expressed when a particular FGF receptor gene is expressed may be used. Then, the
30 nucleic acid will be transcribed when the FGF receptor, such as FGFR1, is expressed,

and not when FGFR2 is expressed. This type of promoter is especially useful when one knows the pattern of FGF receptor expression in a particular tissue, so that specific cells within that tissue may be killed upon transcription of a cytotoxic agent gene without affecting the surrounding tissues.

5 If the domain binds in a sequence specific manner, the construct must contain the sequence that binds to the nucleic acid binding domain. As described below, the target nucleotide sequence may be contained within the coding region of the cytocide, in which case, no additional sequence need be incorporated. Additionally, it may be desirable to have multiple copies of target sequence. If the target sequence is
10 coding sequence, the additional copies must be located in non-coding regions of the cytocide-encoding agent. The target sequences of the nucleic acid binding domains are typically generally known. If unknown, the target sequence may be readily determined. Techniques are generally available for establishing the target sequence (*e.g.*, *see* PCT Application WO 92/05285 and U.S. Serial No. 586,769).

15

G. Other Elements

1. Nuclear translocation signal

As used herein, a "nuclear translocation or targeting sequence" (NTS) is a sequence of amino acids in a protein that are required for translocation of the protein
20 into a cell nucleus. Examples of NTSs are set forth in Table 2 below. Comparison with known NTSs, and if necessary testing of candidate sequences, should permit those of skill in the art to readily identify other amino acid sequences that function as NTSs. A heterologous NTS refers to an NTS that is different from the NTS that occurs in the wild-type peptide, polypeptide, or protein. For example, the NTS may be derived from
25 another polypeptide, it may be synthesized, or it may be derived from another region in the same polypeptide.

TABLE 2

Source	Sequence*	SEQ ID NO.
SV40 large T	Pro ¹²⁶ LysLysArgLysValGlu	24
Polyoma large T	Pro ²⁷⁹ ProLysLysAlaArgGluVal	25
Human c-Myc	Pro ¹²⁰ AlaAlaLysArgValLysLeuAsp	26
Adenovirus E1A	Lys ²⁸¹ ArgProArgPro	27
Yeast mat α_2	Lys ³ IleProlleLys	28
c-Erb-A	A. Gly ²² LysArgLysArgLysSer	29
	B. Ser ¹²⁷ LysArgValAlaLysArgLysLeu	30
	C. Ser ¹⁸¹ HisTrpLysGlnLysArgLysPhe	31
c-Myb	Pro ⁵²¹ LeuLeuLysLysIleLysGln	32
p53	Pro ³¹⁶ GlnProLysLysLysPro	33
Nucleolin	Pro ²⁷⁷ GlyLysArgLysLysGluMetThrLysGlnLysGluValPro	34
HIV Tat	Gly ⁴⁸ ArgLysLysArgArgGlnArgArgArgAlaPro	35
FGF-1	AsnTyrLysLysProLysLeu	36
FGF-2	HisPheLysAspProLysArg	37
FGF-3	AlaProArgArgArgLysLeu	38
FGF-4	IleLysArgLeuArgArg	39
FGF-5	GlyArgArg	—
FGF-6	IleLysArgGlnArgArg	40
FGF-7	IleArgValArgArg	41

*Superscript indicates position in protein

- 5 In order to deliver the nucleic acid to the nucleus, the conjugate should include an NTS. If the conjugate is designed such that the receptor-binding internalized ligand and linked nucleic acid binding domain is cleaved or dissociated in the cytoplasm, then the NTS should be included in a portion of the complex that remains bound to the nucleic acid, so that, upon internalization, the conjugate will be trafficked
- 10 to the nucleus. Thus, the NTS is preferably included in the nucleic acid binding domain, but may additionally be included in the ligand. An NTS is preferred if the cytocide-encoding agent is DNA. If the cytocide-encoding agent is mRNA, an NTS

may be omitted. The nuclear translocation sequence (NTS) may be a heterologous sequence or a may be derived from the selected growth factor. All presently identified members of the FGF family of peptides contain an NTS (*see, e.g.*, International Application WO 91/15229 and Table 2). A typical consensus NTS sequence contains
5 an amino-terminal proline or glycine followed by at least three basic residues in a array of seven to nine amino acids (*see, e.g.*, Dang et al., *J. Biol. Chem.* 264:18019-18023, 1989; Dang et al., *Mol. Cell. Biol.* 8:4049-4058, 1988, and Table 2).

2. Cytoplasm-translocation signal

10 Cytoplasm-translocation signal sequence is a sequence of amino acids in a protein that cause retention of proteins in the lumen of the endoplasmic reticulum and/or translocate proteins to the cytosol. The signal sequence in mammalian cells is KDEL (Lys-Asp-Glu-Leu) (SEQ ID NO. 42) (Munro and Pelham, *Cell* 48:899-907, 1987). Some modifications of this sequence have been made without loss of activity.
15 For example, the sequences RDEL (Arg-Asp-Glu-Leu) (SEQ ID NO. 43) and KEEL (Lys-Glu-Glu-Leu) (SEQ ID NO. 44) confer efficient or partial retention, respectively, in plants (Denecke et al., *Embo. J.* 11:2345-2355, 1992).

A cytoplasm-translocation signal sequence may be included in either the receptor-internalized binding ligand or the nucleic acid binding domain part or both. If
20 cleavable linkers are used to link the ligand with the nucleic acid binding domain, the cytoplasm-translocation signal is preferably included in the nucleic acid binding domain, which will stay bound to the cytocide-encoding agent. Additionally, a cytoplasmic-translocation signal sequence may be included in the receptor-internalized binding ligand, as long as it does not interfere with receptor binding. Similarly, the
25 signal sequence placed in the nucleic acid binding domain should not interfere with binding to the cytocide-encoding agent.

3. Endosome-disruptive peptides

In addition, or alternatively, membrane-disruptive peptides may be
30 incorporated into the complexes. For example, adenoviruses are known to enhance

disruption of endosomes. Virus-free viral proteins, such as influenza virus hemagglutinin HA-2, also disrupt endosomes and are useful in the present invention. Other proteins may be tested in the assays described herein to find specific endosome disrupting agents that enhance gene delivery. In general, these proteins and peptides are
5 amphipathic (*see* Wagner et al., *Adv. Drug. Del. Rev.* 14:113-135, 1994).

Endosome-disruptive peptides, sometimes called fusogenic peptides, may be incorporated into the complex of receptor-internalized binding ligand, nucleic acid binding domain, and cytocide-encoding agent. Two such peptides derived from influenza virus are: GLFEAIEGFIENGWEGMIDGGGC (SEQ. ID NO. 45) and
10 GLFEAIEGFIENGWEGMIDGWYGC (SEQ. ID NO. 46). Other peptides useful for disrupting endosomes may be identified by general characteristics: 25-30 residues in length, contain an alternating pattern of hydrophobic domains and acidic domains, and at low pH (*e.g.*, pH 5) form amphipathic α -helices. A candidate endosome-disrupting peptide is tested by incorporating it into the complex and determining whether it
15 increases the total number of cells expressing the target gene. The peptides are added to a complex having excess negative charge. For example, a DNA construct is complexed with an FGF-poly-L-lysine chemical conjugate so that only a portion of the negative charge of the DNA is neutralized. Poly-L-lysine is added to further bind the DNA and a fusogenic peptide is then added. Optional ratios of DNA, poly-L-lysine and fusogenic
20 peptide are determined using assays, such as gene expression and cell viability.

The fusogenic peptides may alternatively be incorporated into the complex as a fusion protein with either the ligand or the nucleic acid binding domain or both. The endosome-disruptive peptide may be present as single or multiple copies at the N- or C- terminus of the ligand. A single fusion protein of the endosome-disruptive
25 peptide, nucleic acid binding domain, and receptor-internalized binding ligand may be constructed and expressed. For insertion into a construct, DNA encoding the endosome-disruptive peptide may be synthesized by PCR using overlapping oligonucleotides and incorporating a restriction site at the 5' and 3' end to facilitate cloning. The sequence may be verified by sequence analysis.

4. Linkers

As used herein, a "linker" is an extension that links the receptor-binding internalized ligand or fragment thereof and the nucleic acid binding domain. In certain instances, the linker is used to conjugate the ligand directly to the nucleic acid. The
5 linkers provided herein confer specificity, enhance intracellular availability, serum stability and/or solubility on the conjugate and may serve to promote condensation of the nucleic acid.

The linkers provided herein confer specificity and serum stability on the cytotoxic conjugate, for example, by conferring specificity for certain proteases,
10 particularly proteases that are present in only certain subcellular compartments or that are present at higher levels in tumor cells than normal cells. Specificity for proteases present in intracellular compartments and absent in blood is particularly preferred. The linkers may also include sorting signals that direct the conjugate to particular intracellular loci or compartments. Additionally, the linkers may reduce steric
15 hindrance between the growth factor and other protein or linked nucleic acid by distancing the components of the conjugate. Linkers may also condense the nucleic acid. For this purpose, the linker comprises highly basic amino acids (*e.g.*, Lys, Arg) and may even by poly-L-lysine.

In order to increase the serum stability, solubility and/or intracellular
20 concentration or condense the targeted agent, one or more linkers (are) inserted between the receptor-binding internalized ligand and the nucleic acid binding domain. These linkers include peptide linkers, such as intracellular protease substrates, and chemical linkers, such as acid labile linkers, ribozyme substrate linkers and others. Peptides linkers may be inserted using heterobifunctional reagents, described below, or,
25 preferably, are linked to FGF, other growth factors, including heparin-binding growth factors, or cytokines by linking DNA encoding the ligand to the DNA encoding the nucleic acid binding domain.

Chemical linkers may be inserted by covalently coupling the linker to the FGF, other growth factor protein, or cytokine and the nucleic acid binding domain. The
30 linker may be bound via the N- or C-terminus or an internal residue. The

heterobifunctional agents, described below, may be used to effect such covalent coupling.

a. Protease substrates

5 Peptides encoding protease-specific substrates may be introduced between the ligand and the nucleic acid binding domain. The peptides may be inserted using heterobifunctional reagents, as described below, or preferably inserted by recombinant means and expression of the resulting chimera.

Any protease specific substrate (*see, e.g., O'Hare et al., FEBS 273:200-204, 1990; Forsberg et al., J. Protein Chem. 10:517-526, 1991; Westby et al., Bioconjugate Chem. 3:375-381, 1992*) may be introduced as a linker as long as the substrate is cleaved in an intracellular compartment. Preferred substrates include those that are specific for proteases that are expressed at higher levels in tumor cells, that are preferentially expressed in the endosome, or that are absent in blood. The following
15 substrates are among those contemplated for use in accord with the methods herein: cathepsin B substrate, cathepsin D substrate, trypsin substrate, thrombin substrate, and recombinant subtilisin substrate.

b. Flexible linkers and linkers that increase the solubility of the conjugates

20 Flexible linkers, which reduce steric hindrance, and linkers that increase solubility of the conjugates are contemplated for use, either alone or with other linkers, such as the protease specific substrate linkers. Typically, these linkers are simple polymers of small amino acids (*i.e., small side groups*) with uncharged polar side
25 groups. These amino acids (Gly, Ser, Thr, Cys, Tyr, Asn, Gln) are more soluble in water. Of these amino acids, Gly and Ser are preferred. Such linkers include, but are not limited to, (Gly₄Ser)_n, (Ser₄Gly)_n and (AlaAlaProAla)_n in which n is 1 to 6, preferably 1-4, such as:

a. Gly₄Ser SEQ ID NO: 47

30 CCATGGGCGG CGGCGGCTCT GCCATGG

b. (Gly₄Ser)₂ SEQ ID NO: 48

CCATGGGCGG CGGCGGCTCT GGCGGCGGCG GCTCTGCCAT GG

c. (Ser₄Gly)₄ SEQ ID NO: 49

CCATGGCCTC GTCGTCGTCG GGCTCGTCGT CGTCGGGCTC GTCGTCGTCG GGCTCGTCGT

5 CGTCGGGCGC CATGG

d. (Ser₄Gly)₂ SEQ ID NO: 50

CCATGGCCTC GTCGTCGTCG GGCTCGTCGT CGTCGGGCGC CATGG

e. (AlaAlaProAla)_n, where n is 1 to 4, preferably 2 (see
SEQ ID NO: 51)

10

c. Heterobifunctional cross-linking reagents

Numerous heterobifunctional cross-linking reagents that are used to form covalent bonds between amino groups and thiol groups and to introduce thiol groups into proteins, are known to those of skill in this art (*see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; see also, e.g., Cumber et al., Bioconjugate Chem. 3:397-401, 1992; Thorpe et al., Cancer Res. 47:5924-5931, 1987; Gordon et al., Proc. Natl. Acad. Sci. 84:308-312, 1987; Walden et al., J. Mol. Cell Immunol. 2:191-197, 1986; Carlsson et al., Biochem. J. 173:723-737, 1978; Mahan et al., Anal. Biochem. 162:163-170, 1987; Wawryznaczk et al., Br. J. Cancer 66:361-366, 1992; Fattom et al., Infection & Immun. 60:584-589, 1992*). These reagents may be used to form covalent bonds between the receptor-binding internalized ligands with protease substrate peptide linkers and nucleic acid binding domain. These reagents include, but are not limited to: N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP; disulfide linker); sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate (sulfo-LC-SPDP); succinimidyl oxycarbonyl- α -methyl benzyl thiosulfate (SMBT, hindered disulfate linker); succinimidyl 6-[3-(2-pyridyldithio) propionamido]hexanoate (LC-SPDP); sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC); succinimidyl 3-(2-pyridyldithio)butyrate (SPDB; hindered disulfide bond linker); sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide) ethyl-1,3'-dithiopropionate (SAED); sulfosuccinimidyl 7-azido-4-methylcoumarin-3-acetate (SAMCA); sulfosuccinimidyl

15

20

25

30

6-[alpha-methyl-alpha-(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-SMPT);
 1,4-di-[3'-(2'-pyridyldithio)propionamido]butane (DPDPB);
 4-succinimidylloxycarbonyl- -methyl- -(2-pyridylthio)toluene (SMPT, hindered disulfate
 linker); sulfosuccinimidyl6[-methyl- -(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-
 5 SMPT); *m*-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS); *m*-maleimidobenzoyl-
 N-hydroxysulfosuccinimide ester (sulfo-MBS); N-succinimidyl(4-
 iodoacetyl)aminobenzoate (SIAB; thioether linker); sulfosuccinimidyl(4-iodoacetyl)amino
 benzoate (sulfo-SIAB); succinimidyl4(*p*-maleimidophenyl)butyrate (SMPB); sulfosuccini-
 midyl4-(*p*-maleimidophenyl)butyrate (sulfo-SMPB); azidobenzoyl hydrazide (ABH).

10 These linkers should be particularly useful when used in combination
 with peptide linkers, such as those that increase flexibility.

d. Acid cleavable, photocleavable, and heat sensitive linkers

Acid cleavable linkers include, but are not limited to,
 15 bismaleimideoxy propane, adipic acid dihydrazide linkers (*see, e.g., Fattom et al.,
 Infection & Immun. 60:584-589, 1992*) and acid labile transferrin conjugates that
 contain a sufficient portion of transferrin to permit entry into the intracellular transferrin
 cycling pathway (*see, e.g., Welhöner et al., J. Biol. Chem. 266:4309-4314, 1991*).
 Conjugates linked via acid cleavable linkers should be preferentially cleaved in acidic
 20 intracellular compartments, such as the endosome.

Photocleavable linkers are linkers that are cleaved upon exposure to light
 (*see, e.g., Goldmacher et al., Bioconj. Chem. 3:104-107, 1992*), thereby releasing the
 targeted agent upon exposure to light. (Hazum et al., *Proc. Eur. Pept. Symp., 16th,*
 Brunfeldt, K (Ed), pp. 105-110, 1981; nitrobenzyl group as a photocleavable protective
 25 group for cysteine; Yen et al., *Makromol. Chem 190:69-82, 1989*; water soluble
 photocleavable copolymers, including hydroxypropylmethacrylamide copolymer,
 glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; and Senter
 et al., *Photochem. Photobiol. 42:231-237, 1985*; nitrobenzyloxycarbonyl chloride cross
 linking reagents that produce photocleavable linkages). Such linkers are particularly
 30 useful in treating dermatological or ophthalmic conditions and other tissues, such as

blood vessels during angioplasty in the prevention or treatment of restenosis, that can be exposed to light using fiber optics. After administration of the conjugate, the eye or skin or other body part can be exposed to light, resulting in release of the targeted moiety from the conjugate. This should permit administration of higher dosages of such
5 conjugates compared to conjugates that release a cytotoxic agent upon internalization. Heat sensitive linkers would also have similar applicability.

H. Expression vectors and host cells for expression of receptor-binding internalized ligands and nucleic acid binding domains

10 Host organisms include those organisms in which recombinant production of heterologous proteins have been carried out, such as bacteria (for example, *E. coli*), yeast (for example, *Saccharomyces cerevisiae* and *Pichia pastoris*), mammalian cells, and insect cells. Presently preferred host organisms are *E. coli* bacterial strains.

15 The DNA construct encoding the desired protein is introduced into a plasmid for expression in an appropriate host. In preferred embodiments, the host is a bacterial host. The sequence encoding the ligand or nucleic acid binding domain is preferably codon-optimized for expression in the particular host. Thus, for example, if human FGF-2 is expressed in bacteria, the codons would be optimized for bacterial
20 usage. For small coding regions the gene can be synthesized as a single oligonucleotide. For larger proteins, splicing of multiple oligonucleotides, mutagenesis, or other techniques known to those in the art may be used. For example, the sequence of a bacterial-codon preferred FGF-SAP fusion is shown in SEQ. ID NO. 80. The sequences of nucleotides in the plasmids that are regulatory regions, such as promoters
25 and operators, are operationally associated with one another for transcription. The sequence of nucleotides encoding the growth factor or growth factor-chimera may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor protein. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium.

30 In preferred embodiments, the DNA plasmids also include a transcription terminator sequence. As used herein, a "transcription terminator region" has either (a) a

subsegment that encodes a polyadenylation signal and polyadenylation site in the transcript, and/or (b) a subsegment that provides a transcription termination signal that terminates transcription by the polymerase that recognizes the selected promoter. The entire transcription terminator may be obtained from a protein-encoding gene, which
5 may be the same or different from the inserted gene or the source of the promoter. Transcription terminators are optional components of the expression systems herein, but are employed in preferred embodiments.

The plasmids used herein include a promoter in operable association with the DNA encoding the protein or polypeptide of interest and are designed for
10 expression of proteins in a bacterial host. It has been found that tightly regulatable promoters are preferred for expression of saporin. Suitable promoters for expression of proteins and polypeptides herein are widely available and are well known in the art. Inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, but are not limited to, the T7 phage promoter and
15 other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the trp, lpp, and lac promoters, such as the lacUV5, from *E. coli*; the P10 or polyhedron gene promoter of baculovirus/insect cell expression systems (*see, e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784) and inducible promoters from other eukaryotic expression systems. For expression of the proteins such promoters are
20 inserted in a plasmid in operative linkage with a control region such as the lac operon.

Preferred promoter regions are those that are inducible and functional in *E. coli*. Examples of suitable inducible promoters and promoter regions include, but are not limited to: the *E. coli* lac operator responsive to isopropyl β -D-thiogalactopyranoside (IPTG; *see, et al. Nakamura et al., Cell 18:1109-1117, 1979*);
25 the metallothionein promoter metal-regulatory-elements responsive to heavy-metal (*e.g.*, zinc) induction (*see, e.g.*, U.S. Patent No. 4,870,009 to Evans et al.); the phage T7lac promoter responsive to IPTG (*see, e.g.*, U.S. Patent No. 4,952,496; and Studier et al., *Meth. Enzymol.* 185:60-89, 1990) and the TAC promoter.

The plasmids also preferably include a selectable marker gene or genes
30 that are functional in the host. A selectable marker gene includes any gene that confers

a phenotype on bacteria that allows transformed bacterial cells to be identified and selectively grown from among a vast majority of untransformed cells. Suitable selectable marker genes for bacterial hosts, for example, include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r) and the kanamycin resistance gene (Kan^r). The kanamycin resistance gene is presently preferred.

The plasmids may also include DNA encoding a signal for secretion of the operably linked protein. Secretion signals suitable for use are widely available and are well known in the art. Prokaryotic and eukaryotic secretion signals functional in *E. coli* may be employed. The presently preferred secretion signals include, but are not limited to, those encoded by the following *E. coli* genes: ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase, and the like (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). In addition, the bacterial pelB gene secretion signal (Lei et al., *J. Bacteriol.* 169:4379, 1987), the phoA secretion signal, and the cek2 functional in insect cell may be employed. The most preferred secretion signal is the *E. coli* ompA secretion signal. Other prokaryotic and eukaryotic secretion signals known to those of skill in the art may also be employed (see, e.g., von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Using the methods described herein, one of skill in the art can substitute secretion signals that are functional in either yeast, insect or mammalian cells to secrete proteins from those cells.

Particularly preferred plasmids for transformation of *E. coli* cells include the pET expression vectors (see U.S. patent 4,952,496; available from Novagen, Madison, WI; see also literature published by Novagen describing the system). Such plasmids include pET 11a, which contains the T7lac promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; pET 12a-c, which contains the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal; and pET 15b (Novagen, Madison, WI), which contains a His-TagTM leader sequence for use in purification with a His column and a thrombin cleavage site that permits cleavage following purification over the column, the T7-lac promoter region and the T7 terminator.

Other preferred plasmids include the pKK plasmids, particularly pKK 223-3, which contains the tac promoter, (available from Pharmacia; *see also* Brosius et al., *Proc. Natl. Acad. Sci.* 81:6929, 1984; Ausubel et al., *Current Protocols in Molecular Biology*; U.S. Patent Nos. 5,122,463, 5,173,403, 5,187,153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, and 5,229,279). Plasmid pKK has been modified by replacement of the ampicillin resistance marker gene, by digestion with *EcoRI*, with a kanamycin resistance cassette with *EcoRI* sticky ends (purchased from Pharmacia; obtained from pUC4K, *see, e.g.*, Vieira et al. (*Gene* 19:259-268, 1982; and U.S. Patent No. 4,719,179). Baculovirus vectors, such as pBlueBac (also called pJVETL and derivatives thereof), particularly pBlueBac III, (*see, e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may also be used for expression of the polypeptides in insect cells. The pBlueBacIII vector is a dual promoter vector and provides for the selection of recombinants by blue/white screening as this plasmid contains the β -galactosidase gene (*lacZ*) under the control of the insect recognizable ETL promoter and is inducible with IPTG. A DNA construct may be made in baculovirus vector pBluebac III and then co-transfected with wild type virus into insect cells *Spodoptera frugiperda* (sf9 cells; *see, e.g.*, Luckow et al., *Bio/technology* 6:47-55, 1988, and U.S. Patent No. 4,745,051).

Other plasmids include the pIN-IIIompA plasmids (*see* U.S. Patent No. 4,575,013; *see also* Duffaud et al., *Meth. Enz.* 153:492-507, 1987), such as pIN-IIIompA2. The pIN-IIIompA plasmids include an insertion site for heterologous DNA linked in transcriptional reading frame with four functional fragments derived from the lipoprotein gene of *E. coli*. The plasmids also include a DNA fragment coding for the signal peptide of the ompA protein of *E. coli*, positioned such that the desired polypeptide is expressed with the ompA signal peptide at its amino terminus, thereby allowing efficient secretion across the cytoplasmic membrane. The plasmids further include DNA encoding a specific segment of the *E. coli* lac promoter-operator, which is positioned in the proper orientation for transcriptional expression of the desired polypeptide, as well as a separate functional *E. coli* lacI gene encoding the associated

repressor molecule that, in the absence of lac operon inducer, interacts with the lac promoter-operator to prevent transcription therefrom. Expression of the desired polypeptide is under the control of the lipoprotein (lpp) promoter and the lac promoter-operator, although transcription from either promoter is normally blocked by the repressor molecule. The repressor is selectively inactivated by means of an inducer molecule thereby inducing transcriptional expression of the desired polypeptide from both promoters.

Preferably, the DNA fragment is replicated in bacterial cells, preferably in *E. coli*. The preferred DNA fragment also includes a bacterial origin of replication, to ensure the maintenance of the DNA fragment from generation to generation of the bacteria. In this way, large quantities of the DNA fragment can be produced by replication in bacteria. Preferred bacterial origins of replication include, but are not limited to, the fl-ori and col E1 origins of replication. Preferred hosts contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (see U.S. Patent No. 4,952,496). Such hosts include, but are not limited to, lysogens *E. coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3). Strain BL21(DE3) is preferred. The pLys strains provide low levels of T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

The DNA fragments provided may also contain a gene coding for a repressor protein. The repressor protein is capable of repressing the transcription of a promoter that contains sequences of nucleotides to which the repressor protein binds. The promoter can be derepressed by altering the physiological conditions of the cell. For example, the alteration can be accomplished by adding to the growth medium a molecule that inhibits the ability to interact with the operator or with regulatory proteins or other regions of the DNA or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive λ cI857 repressor, and the like. The *E. coli* lacI repressor is preferred.

DNA encoding full-length FGF-2 or FGF-2 mutein is linked to DNA encoding an nucleic acid binding domain, such as protamine, and introduced into the pET vectors, including pET-11a and pET-12a expression vectors (Novagen, Madison, WI), for intracellular and periplasmic expression, respectively, of FGF-protamine fusion proteins.

I. Preparation of complexes containing receptor-binding internalized ligands/nucleic acid binding domain conjugates and cytocide-encoding agents

Within the context of this invention, specificity of delivery is achieved through the ligand. Typically, a nucleic acid binding domain is coupled to a receptor-binding internalized ligand, either by chemical conjugation or as a fusion protein. As described below, the ligand may alternatively be coupled directly to the nucleic acid and then complexed with a nucleic acid binding protein, such as poly-lysine, which serves to condense the nucleic acid. Linkers as described above may optionally be used. The receptor-binding internalized ligand confers specificity of delivery in a cell-specific manner. The choice of the receptor-binding internalized ligand to use will depend upon the receptor expressed by the target cells. The receptor type of the target cell population may be determined by conventional techniques such as antibody staining, PCR of cDNA using receptor-specific primers, and biochemical or functional receptor binding assays. It is preferable that the receptor be cell type-specific or have increased expression or activity (*i.e.*, higher rate of internalization) within the target cell population.

As described herein, the nucleic acid binding domain can be of two types, non-specific in its ability to bind nucleic acid, or highly specific so that the amino acid residues bind only the desired nucleic acid sequence. Nonspecific binding proteins, polypeptides, or compounds are generally polycationic or highly basic. Lys and Arg are the most basic of the 20 common amino acids; proteins enriched for these residues are candidates for nucleic acid binding domains. Examples of basic proteins include histones, protamines, and repeating units of lysine and arginine. Poly-L-lysine is an often-used nucleic acid binding domain (*see* U.S. Patent Nos. 5,166,320 and 5,354,844). Poly-L-lysine and protamine are preferred. Other polycations, such as

spermine and spermidine, may also be used to bind nucleic acids. By way of example, the sequence-specific proteins, including gal4, Sp-1, AP-1, myoD and the *rev* gene product from HIV, may be used. Specific nucleic acid binding domains can be cloned in tandem, individually, or multiply to a desired region of the receptor-binding
5 internalized ligand of interest. Alternatively, the ligand and binding domain can be chemically conjugated to each other.

The corresponding sequence that binds a sequence-specific domain is incorporated into the construct to be delivered. Complexing the cytotoxic-encoding agent to the receptor-binding internalized ligand/nucleic acid binding domain allows
10 specific binding to the nucleic acid binding domain. Even greater specificity of binding may be achieved by identifying and using the minimal amino acid sequence that binds to the cytotoxic-encoding agent of interest. For example, phage display methods can be used to identify amino acids residues of varying length that will bind to specific nucleic acid sequences with high affinity. (See U.S. Patent No. 5,223,409.) The peptide
15 sequence can then be cloned into the receptor-binding internalized ligand as a single copy or multiple copies. Alternatively, the peptide may be chemically conjugated to the receptor-binding internalized ligand. Incubation of the cytotoxic-encoding agent with the conjugated proteins will result in a specific binding between the two.

These complexes may be used to deliver nucleic acids that encode
20 saporin, other cytotoxic proteins, or prodrugs into cells with appropriate receptors that are expressed, over-expressed or more active in internalization upon binding. The cytotoxic gene is cloned downstream of a mammalian promoter such as *c-myc*, SV40 early or late gene, CMV-IE, TK or adenovirus promoter. As described above, promoters of interest may be active in any cell type, active only in a tissue-specific
25 manner, such as α -crystalline or tyrosinase, event specific, or inducible, such as the MMTV LTR.

1. Chemical conjugation

a. Preparation of receptor-binding internalized ligands

Receptor-binding internalized ligands are prepared as discussed by any suitable method, including recombinant DNA technology, isolation from a suitable source, purchase from a commercial source, or chemical synthesis. The selected linker or linkers is (are) linked to the receptor-binding internalized ligands by chemical reaction, generally relying on an available thiol or amine group on the receptor-binding internalized ligands. Heterobifunctional linkers are particularly suited for chemical conjugation. Alternatively, if the linker is a peptide linker, then the receptor-binding internalized ligands, linker and nucleic acid binding domain can be expressed recombinantly as a fusion protein.

Any protein that binds and internalizes through a receptor interaction may be used herein. In particular, any member of the FGF family of peptides or portion thereof that binds to an FGF receptor and internalizes a linked agent may be used herein. For the chemical conjugation methods the protein may be produced recombinantly, produced synthetically or obtained from commercial or other sources. For the preparation of fusion proteins, the DNA encoding the FGF may be obtained from any known source or synthesized according to its DNA or amino acid sequences (see discussion above).

Although any of the growth factors may be conjugated in this manner, FGF, VEGF, and HBEGF conjugation are discussed merely by way of example and not by way of limitation.

If necessary or desired, the heterogeneity of preparations of ligand (e.g., FGF) containing chemical conjugates and fusion proteins can be reduced by modifying the ligand by deleting or replacing a site(s) that causes the heterogeneity. Such sites in FGF are typically cysteine residues that upon folding of the protein remain available for interaction with other cysteines or for interaction with more than one cytotoxic molecule per molecule of FGF peptide. Thus, such cysteine residues do not include any cysteine residue that is required for proper folding of the FGF peptide or for binding to an FGF receptor and internalization. For chemical conjugation, one cysteine residue

that in physiological conditions is available for interaction is not replaced but is used as the site for linking the cytotoxic moiety. The resulting modified FGF is thus conjugated with a single species of nucleic acid binding domain (or nucleic acid).

The polypeptide reactive with an FGF receptor may be modified by
5 removing one or more reactive cysteines that are not required for receptor binding, but that are available for reaction with appropriately derivatized cytotoxic agent, so that the resulting FGF protein has only one cysteine residue available for conjugation with the cytotoxic agent. If necessary, the contribution of each cysteine to the ability to bind to FGF receptors may be determined empirically. Each cysteine residue may be
10 systematically replaced with a conservative amino acid change (*see* Table 1, above) or deleted. The resulting mutein is tested for the requisite biological activity, the ability to bind to FGF receptors and internalize linked cytotoxic moieties. If the mutein retains at least 50% of wild-type activity, then the cysteine residue is not required. Additional cysteines are systematically deleted and replaced and the resulting muteins are tested for
15 activity. In this manner the minimum number and identity of the cysteines needed to retain the ability to bind to an FGF receptor and internalize may be determined. The resulting mutant FGF is then tested for retention of the ability to target a cytotoxic agent to a cell that expresses an FGF receptor and to internalize the cytotoxic agent into such cells. Retention of proliferative activity is indicative, though not definitive, of the
20 retention of such activities. Proliferative activity may be measured by any suitable proliferation assay, such as the assay, exemplified below, that measures the increase in cell number of bovine aortic endothelial cells.

It is noted, however, that modified or mutant FGFs may exhibit reduced or no proliferative activity, but may be suitable for use herein, if they retain the ability
25 to target cytocide-encoding agent to cells bearing FGF receptors and result in internalization. Certain residues of FGF-2 have been associated with proliferative activity. Modification of these residues arg 116, lys 119, tyr 120, trp 123 to ile 116, glu 119, ala 120, ala 123 may be made individually (*see* SEQ ID NOs. 81-84) to remove this function. The resulting protein is tested for proliferative activity by a standard
30 assay.

Any of FGF-1 - FGF-9 may be used. The complete amino acid sequence of each of FGF-1 - FGF- 9 is known (*see, e.g.*, SEQ ID NO. 10 (FGF-1) and SEQ ID NOs. 12-18 (FGF-3 - FGF-9, respectively)). Comparison among the amino acid sequences of FGF-1 -FGF-9 reveals that one Cys is conserved among FGF family of peptides (*see* Table 3). These cysteine residues may be required for secondary structure and are not preferred residues to be altered. Each of the remaining cysteine residues may be systematically deleted and/or replaced by a serine residue or other residue that would not be expected to alter the structure of the protein. The resulting peptide is tested for biological activity. If the cysteine residue is necessary for retention of biological activity it is not deleted; if it not necessary, then it is preferably replaced with a serine or other residue that should not alter the secondary structure of the resulting protein.

The cysteine residues from each of FGF-1 - FGF-9 that appear to be essential for retention of biological activity and that are not preferred residues for deletion or replacement are as follows:

TABLE 3

FGF-1	cys ⁹⁸
FGF-2	cys ¹⁰¹
FGF-3	cys ¹¹⁵
FGF-4	cys ¹⁵⁵
FGF-5	cys ¹⁶⁰
FGF-6	cys ¹⁴⁷
FGF-7	cys ¹³⁷
FGF-8	cys ¹²⁷
FGF-9	cys ¹³⁴

For example, FGF-1 has cysteines at positions 31, 98 and 132; FGF-2 has cysteines at positions 34, 78, 96 and 101; FGF-3 has cysteines at positions 50 and 115; FGF-4 has cysteines at positions 88 and 155; FGF-5 has cysteines at positions 19,

93, 160 and 202; FGF-6 has cysteines at positions 80 and 147; FGF-7 has cysteines at positions 18, 23, 32, 46, 71, 133 and 137; FGF-8 has cysteines at positions 10, 19, 109 and 127; and FGF-9 has cysteines at positions 68 and 134.

Since FGF-3, FGF-4 and FGF-6 have only two cysteines, for purposes of
5 chemical conjugation, preferably neither cysteine is deleted or replaced, unless another residue, preferably one near either terminus, is replaced with a cysteine. With respect to the other FGF family members, at least one cysteine must remain available for conjugation with the cytotoxic conjugate and probably two cysteines, but at least the cysteine residues set forth in Table 3. A second cysteine may be required to form a
10 disulfide bond. Thus, any FGF peptide that has more than three cysteines is be modified for chemical conjugation by deleting or replacing the other cysteine residues. FGF peptides that have three cysteine residues are modified by elimination of one cysteine, conjugated to a cytotoxic moiety and tested for the ability to bind to FGF receptors and internalize the cytotoxic moiety.

15 In accord with the methods herein, several muteins of basic FGF for chemical conjugation have been produced (preparation of muteins for recombinant expression of the conjugate is described below). DNA, obtained from pFC80 (*see* PCT Application Serial No. PCT/US93/05702; United States Application Serial No. 07/901,718; *see also* SEQ ID NO. 52) encoding basic FGF has been mutagenized.
20 Mutagenesis of cysteine 78 of basic FGF (FGF-2) to serine ([C78S]FGF) or cysteine 96 to serine ([C96S]FGF) produced two mutants that retain virtually complete proliferative activity of native basic FGF as judged by the ability to stimulate endothelial cell proliferation in culture. The activities of the two mutants and the native protein do not significantly differ as assessed by efficacy or maximal response. Sequence analysis of
25 the modified DNA verified that each of the mutants has one codon for cysteine converted to that for serine. The construction and biological activity of FGF-1 with cysteine substitutions of one, two or all three cysteines has been disclosed (U.S. Patent No. 5,223,483). The mitogenic activity of the mutants was similar to or increased over the native protein. Thus, any of the cysteines may be mutated and FGF-1 will still bind
30 and internalize.

The resulting mutein FGF or unmodified FGF is reacted with a nucleic acid binding domain. The bFGF muteins may react with a single species of derivatized nucleic acid binding domain (mono-derivatized nucleic acid binding domain), thereby resulting in monogenous preparations of FGF-nucleic acid binding domain conjugates and homogeneous compositions of FGF-nucleic acid binding domain chemical conjugates. The resulting chemical conjugates do not aggregate and retain the requisite biological activities.

VEGF or HBEGF may be isolated from a suitable source or may be produced using recombinant DNA methodology, discussed below. To effect chemical conjugation herein, the growth factor protein is conjugated generally via a reactive amine group or thiol group to the nucleic acid binding domain directly or through a linker to the nucleic acid binding domain. The growth factor protein is conjugated either via its N-terminus, C-terminus, or elsewhere in the polypeptide. In preferred embodiments, the growth factor protein is conjugated via a reactive cysteine residue to the linker or to the nucleic acid binding domain. The growth factor can also be modified by addition of a cysteine residue, either by replacing a residue or by inserting the cysteine, at or near the amino or carboxyl terminus, within about 20, preferably 10 residues from either end, and preferably at or near the amino terminus.

In certain embodiments, the heterogeneity of preparations may be reduced by mutagenizing the growth factor protein to replace reactive cysteines, leaving, preferably, only one available cysteine for reaction. The growth factor protein is modified by deleting or replacing a site(s) on the growth factor that causes the heterogeneity. Such sites are typically cysteine residues that, upon folding of the protein, remain available for interaction with other cysteines or for interaction with more than one cytotoxic molecule per molecule of heparin-binding growth factor peptide. Thus, such cysteine residues do not include any cysteine residue that are required for proper folding of the growth factor or for retention of the ability to bind to a growth factor receptor and internalize. For chemical conjugation, one cysteine residue that, in physiological conditions, is available for interaction, is not replaced because it is

used as the site for linking the cytotoxic moiety. The resulting modified heparin-binding growth factor is conjugated with a single species of cytotoxic conjugate.

Alternatively, the contribution of each cysteine to the ability to bind to VEGF, HBEGF or other heparin-binding growth factor receptors may be determined empirically as described herein. Each cysteine residue may be systematically replaced with a conservative amino acid change (*see* Table 1, above) or deleted. The resulting mutein is tested for the requisite biological activity: the ability to bind to growth factor receptors and internalize linked nucleic acid binding domain and agents. If the mutein retains this activity, then the cysteine residue is not required. Additional cysteines are systematically deleted and replaced and the resulting muteins are tested for activity. Each of the remaining cysteine residues may be systematically deleted and/or replaced by a serine residue or other residue that would not be expected to alter the structure of the protein. The resulting peptide is tested for biological activity. If the cysteine residue is necessary for retention of biological activity it is not deleted; if it not necessary, then it is preferably replaced with a serine or other residue that should not alter the secondary structure of the resulting protein. In this manner the minimum number and identity of the cysteines needed to retain the ability to bind to a heparin-binding growth factor receptor and internalize may be determined. It is noted, however, that modified or mutant heparin-binding growth factors may exhibit reduced or no proliferative activity, but may be suitable for use herein, if they retain the ability to target a linked cytotoxic agent to cells bearing receptors to which the unmodified heparin-binding growth factor binds and result in internalization of the cytotoxic moiety. In the case of VEGF, VEGF₁₂₁ contains 9 cysteines and each of VEGF₁₆₅, VEGF₁₈₉ and VEGF₂₀₆ contain 7 additional residues in the region not present in VEGF₁₂₁. Any of the 7 are likely to be non-essential for targeting and internalization of linked cytotoxic agents. Recently, the role of Cys-25, Cys-56, Cys-67, Cys-101, and Cys-145 in dimerization and biological activity was assessed (Claffery et al., *Biochem. Biophys. Acta* 1246:1-9, 1995). Dimerization requires Cys-25, Cys-56, and Cys-67. Substitution of any one of these cysteine residues resulted in secretion of a monomeric VEGF, which was inactive in both vascular permeability and endothelial cell mitotic assays. In contrast, substitution

of Cys 145 had no effect on dimerization, although biological activities were somewhat reduced. Substitution of Cys-101 did not result in the production of a secreted or cytoplasmic protein. Thus, substitution of Cys-145 is preferred.

The VEGF monomers are preferably linked via non-essential cysteine
5 residues to the linkers or to the targeted agent. VEGF that has been modified by introduction of a Cys residue at or near one terminus, preferably the N-terminus is preferred for use in chemical conjugation. For use herein, preferably the VEGF is dimerized prior to linkage to the linker and/or targeted agent. Methods for coupling proteins to the linkers, such as the heterobifunctional agents, or to nucleic acids, or to
10 proteins are known to those of skill in the art and are also described herein.

For recombinant expression using the methods described herein, up to all cysteines in the HBEGF polypeptide that are not required for biological activity can be deleted or replaced. Alternatively, for use in the chemical conjugation methods herein, all except one of these cysteines, which will be used for chemical conjugation to the
15 cytotoxic agent, can be deleted or replaced. Each of the HBEGF polypeptides described herein have six cysteine residues. Each of the six cysteines may independently be replaced and the resulting mutein tested for the ability to bind to HBEGF receptors and to be internalized. Alternatively, the resulting mutein-encoding DNA is used as part of a construct containing DNA encoding the nucleic acid binding domain linked to the
20 HBEGF-encoding DNA. The construct is expressed in a suitable host cell and the resulting protein tested for the ability to bind to HBEGF receptors and internalize. As long as this ability is retained the mutein is suitable for use herein.

Methods for chemical conjugation of proteins are known to those of skill in the art. The preferred methods for chemical conjugation depend on the selected
25 components, but preferably rely on disulfide bond formation. For example, if the targeted agent is SPDP-derivatized saporin, then it is advantageous to dimerize the VEGF moiety prior coupling or conjugating to the derivatized saporin. If VEGF is modified to include a cysteine residue at or near the N-, preferably, or C- terminus, then dimerization should follow coupling to the nucleic acid binding domain. To effect

chemical conjugation herein, the HBEGF polypeptide is linked via one or more selected linkers or directly to the nucleic acid binding domain.

5 b. Preparation of nucleic acid binding domains for chemical conjugation

A nucleic acid binding domain is prepared for chemical conjugation. For chemical conjugation, a nucleic acid binding domain may be derivatized with SPDP or other suitable chemicals. If the binding domain does not have a Cys residue available for reaction, one can be either inserted or substituted for another amino acid. If desired,
10 mono-derivatized species may be isolated, essentially as described.

For chemical conjugation, the nucleic acid binding domain may be derivatized or modified such that it includes a cysteine residue for conjugation to the receptor-binding internalized ligand. Typically, derivatization proceeds by reaction with SPDP. This results in a heterogeneous population. For example, nucleic acid
15 binding domain that is derivatized by SPDP to a level of 0.9 moles pyridine-disulfide per mole of nucleic acid binding domain includes a population of non-derivatized, mono-derivatized and di-derivatized SAP. nucleic acid binding domain proteins, which are overly derivatized with SPDP, may lose ability to bind nucleic acid because of reaction with sensitive lysines (Lambert et al., *Cancer Treat. Res.* 37:175-209, 1988).
20 The quantity of non-derivatized nucleic acid binding domain in the preparation of the non-purified material can be difficult to judge and this may lead to errors in being able to estimate the correct proportion of derivatized nucleic acid binding domain to add to the reaction mixture.

Because of the removal of a negative charge by the reaction of SPDP
25 with lysine, the three species, however, have a charge difference. The methods herein rely on this charge difference for purification of mono-derivatized nucleic acid binding domain by Mono-S cation exchange chromatography. The use of purified mono-derivatized nucleic acid binding domain has distinct advantages over the non-purified material. The amount of receptor-binding internalized ligand that can react with nucleic
30 acid binding domain is limited to one molecule with the mono-derivatized material, and it is seen in the results presented herein that a more homogeneous conjugate is

produced. There may still be sources of heterogeneity with the mono-derivatized nucleic acid binding domain used here but is acceptable as long as binding to the cytocide-encoding agent is not impacted.

Because more than one amino group on the nucleic acid binding domain
5 may react with the succinimidyl moiety, it is possible that more than one amino group on the surface of the protein is reactive. This creates potential for heterogeneity in the mono-derivatized nucleic acid binding domain.

As an alternative to derivatizing to introduce a sulfhydryl, the nucleic acid binding domain can be modified by the introduction of a cysteine residue.
10 Preferred loci for introduction of a cysteine residue include the N-terminus region, preferably within about one to twenty residues from the N-terminus of the nucleic acid binding domain.

Using either methodology (reacting mono-derivatized nucleic acid binding domain or introducing a Cys residue into nucleic acid binding domain), the
15 resulting preparations of chemical conjugates are monogenous; compositions containing the conjugates also appear to be free of aggregates.

2. Fusion protein of receptor-binding internalized ligands and nucleic acid binding domain

20 As a preferred alternative, heterogeneity can be avoided by producing a fusion protein of receptor-binding internalized ligand and nucleic acid binding domain, as described below. Expression of DNA encoding a fusion of a receptor-binding internalized ligand polypeptide linked to the nucleic acid binding domain results in a more homogeneous preparation of cytotoxic conjugates. Aggregate formation can be
25 reduced in preparations containing the fusion proteins by modifying the receptor-binding internalized ligand, such as by removal of nonessential cysteines, and/or the nucleic acid binding domain to prevent interactions between conjugates via free cysteines. Optionally, one or more coding regions for endosome-disruptive peptide may be constructed as part of the fusion protein.

30 DNA encoding the polypeptides may be isolated, synthesized or obtained from commercial sources or prepared as described herein. Expression of

recombinant polypeptides may be performed as described herein; and DNA encoding these polypeptides may be used as the starting materials for the methods herein.

As described above, DNA encoding FGF, VEGF, HBEGF hepatocyte growth factor, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-13, TNF, GM-CSF, IFN and IGF polypeptides and/or the amino acid sequences of these factors are described above. DNA may be prepared synthetically based on the amino acid or DNA sequence or may be isolated using methods known to those of skill in the art, such as PCR, probe hybridization of libraries, and the like or obtained from commercial or other sources. For example, suitable methods are described in the Examples for amplifying FGF encoding cDNA from plasmids containing FGF encoding cDNA.

As described herein, such DNA may then be mutagenized using standard methodologies to delete or replace any cysteine residues that are responsible for aggregate formation. If necessary, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or replacing a cysteine residue and ascertaining whether the resulting growth factor with the deleted cysteine forms aggregates in solutions containing physiologically acceptable buffers and salts. Loci for insertion of cysteine residues may also be determined empirically. Generally, regions at or near (within 20, preferably 10 amino acids) the C- or, preferably, the N-terminus are preferred.

The DNA construct encoding the fusion protein can be inserted into a plasmid and expressed in a selected host, as described above, to produce a recombinant receptor-binding internalized ligand—nucleic acid binding domain conjugate. Multiple copies of the chimera can be inserted into a single plasmid in operative linkage with one promoter. When expressed, the resulting protein will then be a multimer. Typically, two to six copies of the chimera are inserted, preferably in a head to tail fashion, into one plasmid.

a. Preparation of muteins for recombinant production of the fusion protein

Removal of cysteines not required for binding and internalization is preferred for both chemical conjugation and recombinant methods in the chemical

conjugation methods, all except one cysteine, which is necessary for chemical conjugation are deleted or replaced. In practice, it appears that for FGF polypeptides only two cysteines (including each of the cysteine residues set forth in Table 3), and perhaps only the cysteines set forth in Table 3, are required for retention of the requisite biological activity of the FGF peptide. Thus, FGF peptides that have more than two cysteines are modified by replacing the remaining cysteines with serines. The resulting muteins may be tested for the requisite biological activity.

FGF peptides, such as FGF-3, FGF-4 and FGF-6, that have two cysteines can be modified by replacing the second cysteine, which is not listed in Table 3, and the resulting mutein used as part of a construct containing DNA encoding the cytotoxic agent linked to the FGF-encoding DNA. The construct is expressed in a suitable host cell and the resulting protein tested for the ability to bind to FGF receptors and internalize the cytotoxic agent. As exemplified herein, conjugates containing bFGF muteins in which Cys⁷⁸ and Cys⁹⁶ have been replaced with serine residues have been prepared.

b. DNA constructs and expression of the DNA constructs

To produce monogenous preparations of fusion protein, DNA encoding the FGF protein or other receptor-binding internalized ligand is modified so that, upon expression, the resulting FGF portion of the fusion protein does not include any cysteines available for reaction. In preferred embodiments, DNA encoding an FGF polypeptide is linked to DNA encoding a nucleic acid binding domain. The DNA encoding the FGF polypeptide or other receptor-binding internalized ligand is modified in order to remove the translation stop codon and other transcriptional or translational stop signals that may be present and to remove or replace DNA encoding the available cysteines. The DNA is then ligated to the DNA encoding the nucleic acid binding domain polypeptide directly or via a linker region of one or more codons between the first codon of the nucleic acid binding domain and the last codon of the FGF. The size of the linker region may be any length as long as the resulting conjugate binds and is internalized by a target cell. Presently, spacer regions of from about one to about

seventy-five to ninety codons are preferred. The order of the receptor-binding internalized ligand and nucleic acid binding domain in the fusion protein may be reversed. If the nucleic acid binding domain is N-terminal, then it is modified to remove the stop codon and any stop signals.

- 5 As discussed above, any heparin-binding protein, including FGF, VEGF, HBEGF, cytokine, growth factor and the like may be modified and expressed in accord with the methods herein. Binding to an FGF receptor followed by internalization are the only activities required for an FGF protein to be suitable for use herein. All of the FGF proteins induce mitogenic activity in a wide variety of normal diploid mesoderm-
10 derived and neural crest-derived cells and this activity is mediated by binding to an FGF cell surface receptor followed by internalization. A test of such "FGF mitogenic activity", which reflects the ability to bind to FGF receptors and to be internalized, is the ability to stimulate proliferation of cultured bovine aortic endothelial cells (*see, e.g.,* Gospodarowicz et al., *J. Biol. Chem.* 257:12266-12278, 1982; Gospodarowicz et al.,
15 *Proc. Natl. Acad. Sci. USA* 73:4120-4124, 1976).

- If the FGF or other ligand has been modified so as to lack mitogenic activity or other biological activities, binding and internalization may still be readily assayed by any one of the following tests or other equivalent tests. Generally, these tests involve labeling the ligand, incubating it with target cells, and visualizing or
20 measuring intracellular label. For example, briefly, FGF may be fluorescently labeled with FITC or radiolabeled with ^{125}I . Fluorescein-conjugated FGF is incubated with cells and examined microscopically by fluorescence microscopy or confocal microscopy for internalization. When FGF is labeled with ^{125}I , the labeled FGF is incubated with cells at 4°C. Cells are temperature shifted to 37°C and washed with 2 M
25 NaCl at low pH to remove any cell-bound FGF. Label is then counted and thereby measuring internalization of FGF. Alternatively, the ligand can be conjugated with an nucleic acid binding domain by any of the methods described herein and complexed with a plasmid encoding saporin. As discussed below, the complex may be used to transfect cells and cytotoxicity measured.

The DNA encoding the resulting receptor-binding internalized ligand—nucleic acid binding domain can be inserted into a plasmid and expressed in a selected host, as described above, to produce a monogenous preparation. Fusion proteins of FGF-2 and protamine are especially suitable for use in the present invention.

5 Multiple copies of the modified receptor-binding internalized ligand/nucleic acid binding domain chimera can be inserted into a single plasmid in operative linkage with one promoter. When expressed, the resulting protein will be a multimer. Typically two to six copies of the chimera are inserted, preferably in a head to tail fashion, into one plasmid.

10 Merely by way of example, DNA encoding human bFGF-SAP having SEQ ID NO. 52 has been mutagenized as described in the Examples using splicing by overlap extension (SOE). Another preferred coding region is set forth in SEQ ID NO. 53. In both instances, in preferred embodiments, the DNA is modified by replacing the cysteines at positions 78 and 96 with serine. The codons encoding
15 cysteine residues at positions 78 and 96 of FGF were converted to serine codons by SOE. Each application of the SOE method uses two amplified oligonucleotide products, which have complementary ends as primers and which include an altered codon at the locus at which the mutation is desired, to produce a hybrid product. A second amplification reaction that uses two primers that anneal at the non-overlapping
20 ends amplify the hybrid to produce DNA that has the desired alteration.

3. Binding of the receptor-binding internalized ligand/nucleic acid binding domain conjugate to cytocide-encoding agents

The receptor-binding internalized ligand/nucleic acid binding domain is
25 incubated with the cytocide-encoding agent, preferably a linear DNA molecule, to be delivered under conditions that allow binding of the nucleic acid binding domain to the agent. Conditions will vary somewhat depending on the nature of the nucleic acid binding domain, but will typically occur in 0.1M NaCl and 20 mM HEPES or other similar buffer. Alternatively, salt conditions can be varied to increase the packing or
30 condensation of DNA. The extent of binding is preferably tested for each preparation.

After complexing, additional nucleic acid binding domain, such as poly-L-lysine, may be added to further condense the nucleic acid.

Merely by way of example, test constructs have been made and tested. One construct is a chemical conjugate of bFGF and poly-L-lysine. The bFGF molecule is a variant in which the Cys residue at position 96 has been changed to a serine; thus, only the Cys at position 78 is available for conjugation. This bFGF is called FGF2-3. The poly-L-lysine was derivatized with SPDP and coupled to FGF2-3. This FGF2-3/poly-L-lysine conjugate was used to deliver a plasmid able to express the β -galactosidase gene.

10 The ability of a construct to bind nucleic acid molecules may be conveniently assessed by agarose gel electrophoresis. Briefly, a plasmid, such as pSV β , is digested with restriction enzymes to yield a variety of fragment sizes. For ease of detection, the fragments may be labeled with ^{32}P either by filling in of the ends with DNA polymerase I or by phosphorylation of the 5'-end with polynucleotide kinase following dephosphorylation by alkaline phosphatase. The plasmid fragments are then incubated with the receptor-binding internalized ligand/nucleic acid binding domain in this case, FGF2-3/poly-L-lysine in a buffered saline solution, such as 20 mM HEPES, pH 7.3, 0.1M NaCl. The reaction mixture is electrophoresed on an agarose gel alongside similarly digested, but nonreacted fragments. If a radioactive label was incorporated, the gel may be dried and autoradiographed. If no radioactive label is present, the gel may be stained with ethidium bromide and the DNA visualized through appropriate red filters after excitation with UV. Binding has occurred if the mobility of the fragments is retarded compared to the control. In the example case, the mobility of the fragments was retarded after binding with the FGF2-3/poly-L-lysine conjugate. If there is insufficient binding, poly-L-lysine may be additionally added until binding is observed.

 Further testing of the conjugate is performed to show that it binds to the cell surface receptor and is internalized into the cell. It is not necessary that the receptor-binding internalized ligand part of the conjugate retain complete biological activity. For example, FGF is mitogenic on certain cell types. As discussed above, this

activity may not always be desirable. If this activity is present, a proliferation assay is performed. Likewise, for each desirable activity, an appropriate assay may be performed. However, for application of the subject invention, the only criteria that need be met are receptor binding and internalization.

5 Receptor binding and internalization may be measured by the following three assays. (1) A competitive inhibition assay of the complex to cells expressing the appropriate receptor demonstrates receptor binding. (2) Receptor binding and internalization may be assayed by measuring expression of a reporter gene, such as β -gal (*e.g.*, enzymatic activity), in cells that have been transformed with a complex of a
10 plasmid encoding a reporter gene and a conjugate of a receptor-binding internalized ligand and nucleic acid binding domain. This assay is particularly useful for optimizing conditions to give maximal transformation. Thus, the optimum ratio of receptor-binding internalized ligand/nucleic acid binding domain to nucleic acid and the amount of DNA per cell may readily be determined by assaying and comparing the enzymatic
15 activity of β -gal. As such, these first two assays are useful for preliminary analysis and failure to show receptor binding or β -gal activity does not per se eliminate a candidate receptor-binding internalized ligand/nucleic acid binding domain conjugate or fusion protein from further analysis. (3) The preferred assay is a cytotoxicity assay performed on cells transformed with a cytocide-encoding agent bound by receptor-binding
20 internalized ligand/nucleic acid binding domain. While, in general, any cytotoxic molecule may be used, ribosome inactivating proteins are preferred and saporin, or another type I ribosome inactivating protein, is particularly preferred. A statistically significant reduction in cell number demonstrates the ability of the receptor-binding internalized ligand/nucleic acid binding domain conjugate or fusion to deliver nucleic
25 acids into a cell.

4. Conjugation of ligand to nucleic acid and binding to nucleic acid binding domain

As an alternative, the receptor-internalized binding ligand may be
30 conjugated to the nucleic acid, either directly or through a linker. Methods for conjugating nucleic acids, at the 5' ends, 3' ends and elsewhere, to the amino and

carboxyl termini and other sites in proteins are known to those of skill in the art (for a review see, e.g., Goodchild, (1993) In: *Perspectives in Bioconjugate Chemistry*, Mears, Ed., American Chemical Society, Washington, D.C. pp. 77-99). For example, proteins have been linked to nucleic acids using ultraviolet irradiation (Sperling et al. (1978) *Nucleic Acids Res.* 5:2755-2773; Fiser et al. (1975) *FEBS Lett.* 52:281-283), bifunctional chemicals (Bäumert et al. (1978) *Eur. J. Biochem.* 89:353-359; and Oste et al. (1979) *Mol. Gen. Genet.* 168:81-86) and photochemical cross-linking (Vanin et al. (1981) *FEBS Lett.* 124:89-92; Rinke et al. (1980) *J. Mol. Biol.* 137:301-314; Millon et al. (1980) *Eur. J. Biochem.* 110:485-454).

10 In particular, the reagents (N-acetyl-N'-(p-glyoxylylbenzoyl)cystamine and 2-iminothiolane have been used to couple DNA to proteins, such as α -macroglobulin (α_2 M) via mixed disulfide formation (see Cheng et al., *Nucleic Acids Res.* 11:659-669, 1983). N-acetyl-N'-(p-glyoxylylbenzoyl)cystamine reacts specifically with nonpaired guanine residues and, upon reduction, generates a free
15 sulfhydryl group. 2-iminothiolane reacts with proteins to generate sulfhydryl groups that are then conjugated to the derivatized DNA by an intermolecular disulfide interchange reaction. Any linkage may be used provided that the targeted nucleic acid is active upon internalization of the conjugate. Thus, it is expected that cleavage of the linkage may be necessary, although it is contemplated that for some reagents, such as
20 DNA encoding ribozymes linked to promoters or DNA encoding therapeutic agents for delivery to the nucleus, such cleavage may not be necessary.

Thiol linkages, which are preferred, can be readily formed using heterbiofunctional reagents. Amines have also been attached to the terminal 5' phosphate of unprotected oligonucleotides or nucleic acids in aqueous solutions by
25 reacting the nucleic acid with a water-soluble carbodiimide, such as 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) or N-ethyl-N'(3-dimethylaminopropyl)carbodiimidehydrochloride (EDCI), in imidazole buffer at pH 6 to produce the 5'phosphorimidazolide. Contacting the 5'phosphorimidazolide with amine-containing molecules, such as an FGF, and ethylenediamine, results in stable phosphoramidates
30 (see, e.g., Chu et al., *Nucleic Acids Res.* 11:6513-6529, 1983; and WO 88/05077). In

particular, a solution of DNA is saturated with EDC, at pH 6 and incubated with agitation at 4°C overnight. The resulting solution is then buffered to pH 8.5 by adding, for example about 3 volutes of 100 mM citrate buffer, and adding about 5 µg - about 20 µg of an FGF, and agitating the resulting mixture at 4°C for about 48 hours. The unreacted protein may be removed from the mixture by column chromatography using, for example, Sephadex G75 (Pharmacia) using 0.1 M ammonium carbonate solution, pH 7.0 as an eluting buffer. The isolated conjugate may be lyophilized and stored until used.

U.S. Patent No. 5,237,016 provides methods for preparing nucleotides that are bromoacetylated at their 5' termini and reacting the resulting oligonucleotides with thiol groups. Oligonucleotides derivatized at their 5'-termini bromoacetyl groups can be prepared by reacting 5'-aminohexyl-phosphoramidate oligonucleotides with bromoacetic acid-N-hydroxysuccinimide ester as described in U.S. Patent No. 5,237,016. This patent also describes methods for preparing thiol-derivatized nucleotides, which can then be reacted with thiol groups on the selected growth factor. Briefly, thiol-derivatized nucleotides are prepared using a 5'-phosphorylated nucleotide in two steps: (1) reaction of the phosphate group with imidazole in the presence of a diimide and displacement of the imidazole leaving group with cystamine in one reaction step; and reduction of the disulfide bond of the cystamine linker with dithiothreitol (see, also, Orgel et al. ((1986) *Nucl. Acids Res.* 14:651, which describes a similar procedure). The 5'-phosphorylated starting oligonucleotides can be prepared by methods known to those of skill in the art (see, e.g., Maniatis et al. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, p. 122).

The nucleic acid, such as a methylphosphonate oligonucleotide (MP-oligomer), may be derivatized by reaction with SPDP or SMPB. The resulting MP-oligomer may be purified by HPLC and then coupled to an FGF, such as an FGF or FGF mutein, modified by replacement of one or more cysteine residues, as described above. The MP-oligomer (about 0.1 µM) is dissolved in about 40-50 µl of 1:1 acetonitrile/water to which phosphate buffer (pH 7.5, final concentration 0.1 M) and a 1 mg MP-oligomer in about 1 ml phosphate buffered saline is added. The reaction is

allowed to proceed for about 5-10 hours at room temperature and is then quenched with about 15 μ L 0.1 iodoacetamide. FGF-oligonucleotide conjugates can be purified on heparin sepharose Hi Trap columns (1 ml, Pharmacia) and eluted with a linear or step gradient. The conjugate should elute in 0.6 M NaCl.

5 The ligand may be conjugated to the nucleic acid construct encoding the cytocide or cytotoxic agent or may be conjugated to a mixture of oligonucleotides complementary to one strand of the construct. The oligonucleotides are then added to single stranded construct produced by melting a double-stranded construct or grown and isolated as single-stranded. As a general guideline, the oligonucleotides should
10 hybridize at a higher temperature than the construct alone, if a double-stranded construct is used as the starting material. The gaps are filled in by DNA polymerase I to generate a construct with one strand conjugated to ligand and one strand unconjugated. Oligonucleotides conjugated to ligand and complementary to the other strand may be used in addition to generate a mixture of constructs with different strands linked to
15 ligand. Any remaining single stranded plasmid may be digested with a single strand specific endonuclease. The ligand-conjugated constructs are then mixed with a nucleic acid binding domain, such as protamine or polylysine, to effect condensation of the construct for delivery. Optimal ratios of ligand to DNA may be determined experimentally by receptor-mediated transfection of a construct containing a reporter
20 gene.

J. Formulation and administration of pharmaceutical compositions

 The conjugates and complexes provided herein are useful in the treatment and prevention of various diseases, syndromes, and hyperproliferative
25 disorders. As used herein, "treatment" means any manner in which the symptoms of a conditions, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein. As used herein, "amelioration" of the symptoms of a particular disorder by administration of a particular pharmaceutical composition refers to any lessening, whether permanent
30 or temporary, lasting or transient that can be attributed to or associated with

administration of the composition. For example, these conjugates and complexes may be used to treat complications of the eye following laser surgery, glaucoma surgery, and removal of pterygii. Following these treatments, reoccurrence of the problem often ensues due to proliferation of cells in the cornea or eye. The conjugates and complexes
5 inhibit the proliferation of these cells. The conjugates and complexes may be used in general to treat pathophysiological conditions, especially FGF-, VEGF-, or HBEGF-mediated pathophysiological conditions by specifically targeting to cells having corresponding receptors.

As used herein, "FGF-mediated pathophysiological condition" refers to a
10 deleterious condition characterized by or caused by proliferation of cells that are sensitive to FGF mitogenic stimulation. Basic FGF-mediated pathophysiological conditions include, but are not limited to, melanoma, other tumors, rheumatoid arthritis, restenosis, Dupuytren's Contracture and certain complications of diabetes, such as proliferative retinopathy.

15 As used herein, "HBEGF-mediated pathophysiological condition" refers to a deleterious condition characterized by or caused by proliferation of cells that are sensitive to HBEGF mitogenic stimulation. HBEGF-mediated pathophysiological conditions include conditions involving pathophysiological proliferation of smooth muscle cells, such as restenosis, certain tumors, such as solid tumors including breast
20 and bladder tumors, tumors involving pathophysiological expression of EGF receptors, dermatological disorders, such as psoriasis, and ophthalmic disorders involving epithelial cells, such as recurrence of pterygii and secondary lens clouding.

Similarly, tumors and hyperproliferating cells expressing cytokine receptors or growth factor receptors may be eliminated. Such diseases include
25 restenosis, Dupuytren's Contracture, diabetic retinopathies, rheumatoid arthritis, Kaposi's sarcoma, lymphomas, leukemias, tumors such as renal cell carcinoma, colon carcinoma, breast cancer, bladder cancer, disorders with underlying vascular proliferation, such as diseases in the back of the eye (e.g., proliferative vitreoretinopathy, macular degeneration and diabetic retinopathy). For treatment of the
30 back of the eye especially, use of the VEGF-receptor promoter to control expression of

the cytocide or cytotoxic agent is preferred. The conjugates may be used to prevent corneal haze or clouding that results from exposure of the cornea to laser radiation during eye surgery, particularly LRK. The haze or clouding appears to result from fibroblastic keratocyte proliferation in the subepithelial zone following photoablation of the cornea.

The conjugates may be used to treat a "hyperproliferative skin disorder." As used herein, it is a disorder that is manifested by a proliferation of endothelial cells of the skin coupled with an underlying vascular proliferation, resulting in a localized patch of scaly or horny or thickened skin or a tumor of endothelial origin. Such disorders include actinic and atopic dermatitis, toxic eczema, allergic eczema, psoriasis, skin cancers and other tumors, such as Kaposi's sarcoma, angiosarcoma, hemangiomas, and other highly vascularized tumors, and vascular proliferative responses, such as varicose veins.

As well, the conjugates may be used to treat or prevent restenosis, a process and the resulting condition that occurs following angioplasty in which the arteries become reclogged. After treatment of arteries by balloon catheter or other such device, denudation of the interior wall of the vessel occurs, including removal of the endothelial cells that constitute the lining of the blood vessels. As a result of this removal and the concomitant vascular injury, smooth muscle cells (SMCs), which form the blood vessel structure, proliferate and fill the interior of the blood vessel. This process and the resulting condition is restenosis.

Pharmaceutical carriers or vehicles suitable for administration of the conjugates and complexes provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the conjugates and complexes may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

The conjugates and complexes can be administered by any appropriate route, for example, orally, parenterally, including intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration. Preferred modes of administration

depend upon the indication treated. Dermatological and ophthalmologic indications will typically be treated locally; whereas, tumors and restenosis, will typically be treated by systemic, intradermal, or intramuscular modes of administration.

The conjugates and complexes herein may be formulated into
5 pharmaceutical compositions suitable for topical, local, intravenous and systemic application. For the ophthalmic uses herein, local administration, either by topical administration or by injection is preferred. Time release formulations are also desirable. Effective concentrations of one or more of the conjugates and complexes are mixed with a suitable pharmaceutical carrier or vehicle. As used herein an "effective amount"
10 of a compound for treating a particular disease is an amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with the disease. Such amount may be administered as a single dosage or may be administered according to a regimen, whereby it is effective. The amount may cure the disease but, typically, is administered in order to ameliorate the symptoms of the disease. Repeated
15 administration may be required to achieve the desired amelioration of symptoms.

As used herein, "an ophthalmically effective amount" is that amount which, in the composition administered and by the technique administered, provides an amount of therapeutic agent to the involved eye tissues sufficient to prevent or reduce corneal haze following excimer laser surgery, prevent closure of a trabeculectomy,
20 prevent or substantially slow the recurrence of pterygii, and other conditions.

The concentrations or amounts of the conjugates and complexes that are effective requires delivery of an amount, upon administration, that ameliorates the symptoms or treats the disease. Typically, the compositions are formulated for single dosage administration. Therapeutically effective concentrations and amounts may be
25 determined empirically by testing the conjugates and complexes in known *in vitro* and *in vivo* systems, such as those described here; dosages for humans or other animals may then be extrapolated therefrom.

The conjugate is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of
30 undesirable side effects on the patient treated. The conjugates may be delivered as

pharmaceutically acceptable salts, esters or other derivatives of the conjugates include any salts, esters or derivatives that may be readily prepared by those of skill in this art using known methods for such derivatization and that produce compounds that may be administered to animals or humans without substantial toxic effects. It is understood
5 that number and degree of side effects depends upon the condition for which the conjugates and complexes are administered. For example, certain toxic and undesirable side effects are tolerated when treating life-threatening illnesses, such as tumors, that would not be tolerated when treating disorders of lesser consequence. The concentration of conjugate in the composition will depend on absorption, inactivation
10 and excretion rates thereof, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

Preferably, the conjugate and complex are substantially pure. As used herein, "substantially pure" means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer
15 chromatography (TLC), gel electrophoresis, high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to
20 those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.

Typically a therapeutically effective dosage should produce a serum concentration of active ingredient of from about 0.1 ng/ml to about 50-100 µg/ml. The
25 pharmaceutical compositions typically should provide a dosage of from about 0.01 mg to about 100 - 2000 mg of conjugate, depending upon the conjugate selected, per kilogram of body weight per day. For example, for treatment of restenosis a daily dosage of about between 0.05 and 0.5 mg/kg (based on FGF-SAP chemical conjugate or an amount of conjugate provided herein equivalent on a molar basis thereto) should be
30 sufficient. Local application for ophthalmic disorders and dermatological disorders

should provide about 1 ng up to 100 μ g, preferably about 1 ng to about 10 μ g, per single dosage administration. It is understood that the amount to administer will be a function of the conjugate selected, the indication treated, and possibly the side effects that will be tolerated.

5 Therapeutically effective concentrations and amounts may be determined for each application herein empirically by testing the conjugates and complexes in known *in vitro* and *in vivo* systems (e.g., murine, rat, rabbit, or baboon models), such as those described herein; dosages for humans or other animals may then be extrapolated therefrom. Demonstration that the conjugates and complexes prevent or inhibit
10 proliferation of serum stimulated corneal keratocytes or fibroblasts explanted from eyes, as shown herein, and demonstration of any inhibition of proliferation of such tissues in rabbits should establish human efficacy. The rabbit eye model is a recognized model for studying the effects of topically and locally applied drugs (*see, e.g.*, U.S. Patent Nos. 5,288,735, 5,263,992, 5,262,178, 5,256,408, 5,252,319, 5,238,925, 5,165,952; *see also*
15 Mirate et al., *Curr. Eye Res.* 1:491-493, 1981).

 The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by
20 extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that
25 the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

 The conjugates and complexes may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for
30 intracisternal or intraspinal application. Such solutions, particularly those intended for

ophthalmic use, may be formulated as 0.01% -10% isotonic solutions, pH about 5-7, with appropriate salts. The ophthalmic compositions may also include additional components, such as hyaluronic acid. The conjugates and complexes may be formulated as aerosols for topical application (*see, e.g.*, U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923).

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of toxicity such as sodium chloride or dextrose. Parental preparations can be enclosed in ampules, disposable syringes or multiple dose vials made of glass, plastic or other suitable material.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof. Liposomal suspensions may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art.

Upon mixing or addition of the conjugate(s) with the vehicle, the resulting mixture may be a solution, suspension, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the conjugate in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined based upon *in vitro* and/or *in vivo* data, such as the data from the mouse xenograft model for tumors or rabbit ophthalmic model. If necessary, pharmaceutically acceptable salts or other derivatives of the conjugates and complexes may be prepared.

The active materials can also be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action, including viscoelastic materials, such as hyaluronic acid, which is sold under the trademark HEALON (solution of a high molecular weight (MW of about 3 millions) fraction of sodium hyaluronate; manufactured by Pharmacia, Inc. *see, e.g.*, U.S. Patent Nos. 5,292,362, 5,282,851, 5,273,056, 5,229,127, 4,517,295 and 4,328,803), VISCOAT (fluorine-containing (meth)acrylates, such as, 1H,1H,2H,2H-hepta-decafluorodecylmethacrylate; *see, e.g.*, U.S. Patent Nos. 5,278,126, 5,273,751 and 5,214,080; commercially available from Alcon Surgical, Inc.), ORCOLON (*see, e.g.*, U.S. Patent Nos. 5,273,056; commercially available from Optical Radiation Corporation), methylcellulose, methyl hyaluronate, polyacrylamide and polymethacrylamide (*see, e.g.*, U.S. Patent No. 5,273,751). The viscoelastic materials are present generally in amounts ranging from about 0.5 to 5.0%, preferably 1 to 3% by weight of the conjugate material and serve to coat and protect the treated tissues. The compositions may also include a dye, such as methylene blue or other inert dye, so that the composition can be seen when injected into the eye or contacted with the surgical site during surgery.

The conjugates and complexes may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye. Such solutions, particularly those intended for ophthalmic use, may be formulated as 0.01%-10% isotonic solutions, pH about 5-7, with appropriate salts. Suitable ophthalmic solutions are known (*see, e.g.*, U.S. Patent No. 5,116,868, which describes typical compositions of ophthalmic irrigation solutions and solutions for topical application). Such solutions, which have a pH adjusted to about 7.4, contain, for example, 90-100 mM sodium chloride, 4-6 mM dibasic potassium phosphate, 4-6 mM dibasic sodium phosphate, 8-12 mM sodium citrate, 0.5-1.5 mM magnesium chloride, 1.5-2.5 mM calcium chloride, 15-25 mM sodium acetate, 10-20 mM D.L.-sodium β -hydroxybutyrate and 5-5.5 mM glucose.

The conjugates and complexes may be prepared with carriers that protect them against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. For example, the composition may be applied during surgery using a sponge, such as a commercially available surgical sponges (*see, e.g.*, U.S. Patent Nos. 3,956,044 and 4,045,238; available from Weck, Alcon, and Mentor), that has been soaked in the composition and that releases the composition upon contact with the eye. These are particularly useful for application to the eye for ophthalmic indications following or during surgery in which only a single administration is possible. The compositions may also be applied in pellets (such as Elvax pellets(ethylene-vinyl acetate copolymer resin); about 1- 5 µg of conjugate per 1 mg resin) that can be implanted in the eye during surgery.

Ophthalmologically effective concentrations or amounts of one or more of the conjugates and complexes are mixed with a suitable pharmaceutical carrier or vehicle. The concentrations or amounts of the conjugates and complexes that are effective requires delivery of an amount, upon administration, that prevents or substantially reduces corneal clouding, trabeculectomy closure, or pterygii recurrence.

The conjugates and complexes herein are formulated into ophthalmologically acceptable compositions and are applied to the affected area of the eye during or immediately after surgery. In particular, following excimer laser surgery, the composition is applied to the cornea; following trabeculectomy the composition is applied to the fistula; and following removal of pterygii the composition is applied to the cornea. The compositions may also be used to treat pterygii. The conjugates and complexes are applied during and immediately following surgery and may, if possible be applied post-operatively, until healing is complete. The compositions are applied as drops for topical and subconjunctival application or are injected into the eye for intraocular application. The compositions may also be absorbed to a biocompatible

support, such as a cellulosic sponge or other polymer delivery device, and contacted with the affected area.

The ophthalmologic indications herein are typically be treated locally either by the application of drops to the affected tissue(s), contacting with a biocompatible sponge that has absorbed a solution of the conjugates and complexes or by injection of a composition. For the indications herein, the composition will be applied during or immediately after surgery in order to prevent closure of the trabeculectomy, prevent a proliferation of keratocytes following excimer laser surgery, or to prevent a recurrence of pterygii. The composition may also be injected into the affected tissue following surgery and applied in drops following surgery until healing is completed. For example, to administer the formulations to the eye, it can be slowly injected into the bulbar conjunctiva of the eye.

Conjugates and complexes with photocleavable linkers are among those preferred for use in the methods herein. Upon administration of such composition to the affected area of the eye, the eye is exposed to light of a wavelength, typically visible or UV that cleaves the linker, thereby releasing the cytotoxic agent.

If oral administration is desired, the conjugate should be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

Oral compositions will generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, such as microcrystalline cellulose, gum tragacanth and gelatin; an excipient such as starch and

lactose, a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a glidant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, and fruit flavoring.

5 When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The conjugates and complexes can also be administered as a component of an elixir, suspension, syrup,
10 wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

 The active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action,
15 such as cis-platin for treatment of tumors.

 Finally, the compounds may be packaged as articles of manufacture containing packaging material, one or more conjugates and complexes or compositions as provided herein within the packaging material, and a label that indicates the indication for which the conjugate is provided.

20 Many methods have been developed to deliver nucleic acid into cells including retroviral vectors, electroporation, CaPO_4 precipitation and microinjection, but each of these methods has distinct disadvantages. Microinjecting nucleic acid into cells is very time consuming because each cell must be manipulated individually. Retroviral vectors can only hold a limited length of nucleic acid and can activate
25 oncogenes depending upon the insertion site in the target chromosome. Conditions for electroporation and CaPO_4 -mediated transfection are harsh and cause much cell death.

 By comparison, receptor mediated gene delivery as described herein is a more desirable method of selectively targeting toxic genes into cells that have "more active" receptors or that overexpress the specific receptor on the cell surface. A
30 receptor may be more active because it has a higher rate of internalization or higher

cycling rate through the endosome to the cell surface. Advantages of this method over other gene delivery methods include increased specificity of delivery, the absence of nucleic acid length limitations, reduced toxicity, and reduced immunogenicity of the conjugate. These characteristics allow for repeated administration of the material with minimal harm to cells and may allow increased level of expression of the toxic protein. In addition, primary cultures can also be treated using this method.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLES

EXAMPLE 1

ISOLATION OF DNA ENCODING SAPORIN

A. Materials and methods

1. Bacterial Strains

E. coli strain JA221 (lpp⁻ hdsM⁺ trpE5 leuB6 lacY recA1 F'[lacI^q lac⁺ pro⁺]) is publicly available from the American Type Culture Collection (ATCC), Rockville, MD 20852, under the accession number ATCC 33875. (JA221 is also available from the Northern Regional Research Center (NRRL), Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, under the accession number NRRL B-15211; *see also* U.S. Patent No. 4,757,013 to Inouye; and Nakamura et al., *Cell* 18:1109-1117, 1979). Strain INV1 α is commercially available from Invitrogen, San Diego, CA.

2. DNA Manipulations

The restriction and modification enzymes employed herein are commercially available in the U.S. Native saporin and rabbit polyclonal antiserum to saporin were obtained as previously described in Lappi et al., *Biochem. Biophys. Res. Comm.* 129:934-942. Ricin A chain is commercially available from Sigma, Milwaukee,

WI. Antiserum was linked to Affi-gel 10 (Bio-Rad, Emeryville, CA) according to the manufacturer's instructions. Sequencing was performed using the Sequenase kit of United States Biochemical Corporation (version 2.0) according to the manufacturer's instructions. Minipreparation and maxipreparation of plasmids, preparation of
5 competent cells, transformation, M13 manipulation, bacterial media, Western blotting, and ELISA assays were according to Sambrook et al., (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). The purification of DNA fragments was done using the GeneClean II kit (Bio 101) according to the manufacturer's instructions. SDS gel electrophoresis was
10 performed on a Phastsystem (Pharmacia).

Western blotting was accomplished by transfer of the electrophoresed protein to nitrocellulose using the PhastTransfer system, as described by the manufacturer. The antiserum to SAP was used at a dilution of 1:1000. Horseradish peroxidase labeled anti-IgG was used as the second antibody (*see* Davis et al., *Basic*
15 *Methods In Molecular Biology*, New York, Elsevier Science Publishing Co., pp 1-338, 1986).

B. Isolation of DNA encoding saporin

20 1. Isolation of genomic DNA and preparation of polymerase chain reaction (PCR) primers

Saponaria officinalis leaf genomic DNA was prepared as described in Bianchi et al., *Plant Mol. Biol.* 11:203-214, 1988. Primers for genomic DNA amplifications were synthesized in a 380B automatic DNA synthesizer. The primer corresponding to the "sense" strand of saporin 5'-
25 CTGCAGAATTCGCATGGATCCTGCTTCAAT-3' (SEQ ID NO. 54) includes an *EcoR* I restriction site adapter immediately upstream of the DNA codon for amino acid -15 of the native saporin N-terminal leader sequence. The primer 5'-CTGCAGAATTCGCCTCGTTTGACTACTTTG-3' (SEQ ID NO. 55) corresponds to the "antisense" strand of saporin and complements the coding sequence of saporin
30 starting from the last 5 nucleotides of the DNA encoding the carboxyl end of the mature

peptide. Use of this primer introduced a translation stop codon and an *EcoRI* restriction site after the sequence encoding mature saporin.

2. Amplification of DNA encoding saporin

5 Unfractionated *Saponaria officinalis* leaf genomic DNA (1 μ l) was mixed in a final volume of 100 μ l containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 2 mM MgCl₂, 0.2 mM dNTPs, 0.8 μ g of each primer. Next, 2.5 U *Taq* DNA polymerase (Perkin Elmer Cetus) were added and the mixture was overlaid with 30 μ l of mineral oil (Sigma). Incubations were done in a DNA Thermal Cycler
10 (Ericomp). One cycle included a denaturation step (94°C for 1 min), an annealing step (60°C for 2 min), and an elongation step (72°C for 3 min). After 30 cycles, a 10 μ l aliquot of each reaction was run on a 1.5% agarose gel to verify the structure of the amplified product.

The amplified DNA was digested with *EcoRI* and subcloned into *EcoRI*-
15 restricted M13mp18 (New England Biolabs, Beverly, MA; *see also* Yanisch-Perron et al. (1985), "Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors", *Gene* 33:103). Single-stranded DNA from recombinant phages was sequenced using oligonucleotides based on internal points in the coding sequence of saporin (*see* Bennati et al., *Eur. J. Biochem.* 183:465-
20 470, 1989). Nine of the M13mp18 derivatives were sequenced and compared. Of the nine sequenced clones, five had unique sequences, set forth as SEQ ID NOs. 19-23, respectively. The clones were designated M13mp18-G4, -G1, -G2, -G7, and -G9. Each of these clones contains all of the saporin coding sequence and 45 nucleotides of DNA encoding the native saporin N-terminal leader peptide.

25 Saporin DNA sequence was also cloned in the pET11a vector. Briefly, the DNA encoding SAP-6 was amplified by polymerase chain reaction (PCR) from the parental plasmid pZ1B1. The plasmid pZ1B1 contains the DNA sequence for human FGF-2 linked to SAP-6 by a two-amino-acid linker (Ala-Met). PZ1B1 also includes the T7 promoter, lac operator, ribosomal binding site, and T7 terminator present in the pET-
30 11a vector. For SAP-6 DNA amplification, the 5' primer (5' CATATGTGTGTCACATCAATCACATTAGAT 3') (SEQ ID NO. 105),

corresponding to the sense strand of SAP-6, incorporated a *NdeI* restriction enzyme site used for cloning. It also contained a Cys codon at position -1 relative to the start site of the mature protein sequence. No leader sequence was included. The 3' primer (5' CAGGTTTGGATCCTTTACGTT 3') (SEQ ID NO. 106) corresponding to the
5 antisense strand of SAP-6 had a *BamHI* site used for cloning. The amplified DNA was gel-purified and digested with *NdeI* and *BamHI*. The digested SAP-6 DNA fragment was subcloned into the *NdeI/BamHI*-digested pZ1B1. This digestion removed FGF-2 and the 5' portion of SAP-6 (up to nucleotide position 650) from the parental rFGF2-SAP vector (pZ1B1) and replaced this portion with a SAP-6 molecule containing a Cys
10 at position -1 relative to the start site of the native mature SAP-6 protein. The resultant plasmid was designated as pZ50B. pZ50B was transformed into *E. coli* strain NovaBlue for restriction and sequencing analysis. The appropriate clone was then transformed into *E. coli* strain BL21(DE3) for expression and large-scale production.

15 C. Mammalian codon optimization of saporin cDNA.

Mammalian expression plasmids encoding β -galactosidase (β -gal), pSV- β and pNASS- β , were obtained from Clontech (Palo Alto, CA). Plasmid pSV β expresses β -gal from the SV40 early promoter. Plasmid pNASSb is a promoterless mammalian reporter vector containing the β -gal gene.

20 The amino acid sequence for the plant protein saporin (SAP) was reverse translated using mammalian codons. The resulting mammalian optimized cDNA was divided into 4 fragments (designated 5'-3' A-D) for synthesis by PCR using overlapping oligos. To facilitate subcloning of each fragment and piecing together of the entire cDNA, restriction enzyme sites were added to the ends of each fragment, and added or
25 removed within each fragment without changing the corresponding amino acid sequence. In addition, the 5' end of the cDNA was modified to include a Kozak sequence for optimal expression in mammalian cells. Fragments A, B, and D were each synthesized by annealing 4 oligos (2 sense, 2 antisense) with 20 base overlaps and using PCR to fill-in and amplify the fragments. The PCR products were then purified using
30 GeneClean (Bio101), digested with restriction enzymes recognizing the sites in the

primers, and subcloned into pBluescript (SK+) (Stratagene). The sequence of the inserts was verified using Sequenase Version 2.0 (United States Biochemical/Amersham). Fragment C was synthesized in two steps: The 5' and 3' halves of the fragment were independently synthesized by PCR using 2 overlapping oligos. The products of these
5 using 2 reactions were then purified and combined and the full-length fragment C was generated by PCR using the outermost oligos as primers. Full-length fragment C was subcloned into pBluescript for sequencing. Fragments A and B were ligated together in pBluescript at an overlapping *KspI* site. Fragments C and D were ligated together in pBluescript at an overlapping *PvuII* site. Fragments A-B and C-D were then joined in
10 pBluescript at an overlapping *AvaI* site to give the full-length mammalian optimized SAP cDNA. β -gal sequences were excised from the plasmids pNASS- β and pSV- β (Clontech) by digestion with *NotI* and replaced with the synthetic SAP gene, which has *NotI* ends. Orientation of the insert was confirmed by restriction enzyme digestion. Large scale plasmid preparations were performed using Qiagen Maxi 500 columns.

15 The oligos used to synthesize each SAP fragment are (5'-3'):

A1(sense):CGTATCAGGCGGCCGCGCCATGGTGACCTCCATCACCTGGACC
TGGTGAACCCACCGCCGGCC (SEQ ID NO.: 89)

20 A2(antisense):TTGGGGTCCTTCACGTTGTTGCGGATCTTGTCCACGAAGGAGG
AGTACTGGCCGGCGGTGGGGTTCACC (SEQ ID NO.: 90)

A3(sense):AACAACTGAAGGACCCCAACCTGAAGTACGGCGGCACCGACAT
CGCCGTGATCGGCCCCCCTC (SEQ ID NO.: 91)

25 A4(antisense):GTGCCGCGGGAGGACTGGAAGTTGATGCGCAGGAAGTCTCCT
TGGAGGGGGGGCCGATCACGGC (SEQ ID NO.: 92)

B1(sense):CTCCCGCGGCACCGTGTCCCTGGGCCTGAAGCGCGACAACCTGTA
30 CGTGGTGGCCTACCTGGCCATGGACAACAC (SEQ ID NO.: 93)

B2(antisense):GCGGTCAGCTCGGCGGAGGTGATCTCGGACTTGAAGTAGTAGG
CGCGGTTACGTTGGTGTGTCCATGGCCAGGTA (SEQ ID NO.: 94)

5 B3(sense):GCCGAGCTGACCGCCCTGTTCCCTGAGGCCACCACCGCCAACCAG
AAGGCCCTGGAGTACACCGAGGACTACCAGTCC (SEQ ID NO.: 95)

B4(antisense):AGCCCGAGCTCCTTGCGGGACTTGTCGCCCTGGGTGATCTGGG
CGTTCTTCTCGATGGACTGGTAGTCCTCGGTGT (SEQ ID NO.: 96)

10

C1(sense):TATAGAATTCCTCGGGCTGGGCATCGACCTGCTGCTGACCTTCATG
GAGGCCGTGAACAAGAAGGCCCGCGTGG (SEQ ID NO.: 97)

C2(antisense):CGGCGGTCATCTGGATGGCGATCAGCAGGAAGCGGGCCTCGTT
15 CTTACCCACGCGGGCCTTCTTGTTTC (SEQ ID NO.: 98)

C3(sense):CGCCATCCAGATGACCGCCGAGGTGGCCCGCTTCCGCTACATCCA
GAACCTGGTGACCAAGAACTTCCCC (SEQ ID NO.: 99)

20 C4(antisense):GGCGGATCCCAGCTGACCTCGAACTGGATCACCTTGTTGTCGG
AGTCGAACTTGTTGGGGAAGTTCTTGGTCACCA (SEQ ID NO.: 100)

D1(sense):CCGGGATCCGTCAGCTGGCGCAAGATCTCCACCGCCATCTACGGC
GACGCCAAGAACGGCG (SEQ ID NO.: 101)

25

D2(antisense):GCACCTTGCCGAAGCCGAAGTCGTAGTCCTTGTTGAACACGCC
GTTCTTGGCGTCGCCGTAGAT (SEQ ID NO.: 102)

D3(sense):TTCGGCTTCGGCAAGGTGCGCCAGGTGAAGGACCTGCAGATGGGC
30 CTGCTGATGTACC (SEQ ID NO.: 103)

D4(antisense):TGAACGTGGCGGCCGCCTACTTGGGCTTGCCCAGGTACATCAG
CAGGCCCAT (SEQ ID NO.: 104)

5

D. pOMPAG4 Plasmid Construction

M13 mp18-G4 was digested with *EcoR* I, and the resulting fragment was ligated into the *EcoR* I site of the vector pIN-IIIompA2 (see, e.g., see, U.S. Patent No. 4,575,013 to Inouye; and Duffaud et al., *Meth. Enz.* 153:492-507, 1987) using the
10 methods described herein. The ligation was accomplished such that the DNA encoding saporin, including the N-terminal extension, was fused to the leader peptide segment of the bacterial ompA gene. The resulting plasmid pOMPAG4 contains the *lpp* promoter (Nakamura et al., *Cell* 18:1109-1117, 1987), the *E. coli* lac promoter operator sequence (*lac O*) and the *E. coli* ompA gene secretion signal in operative association with each
15 other and with the saporin and native N-terminal leader-encoding DNA listed in SEQ ID NO. 19. The plasmid also includes the *E. coli* lac repressor gene (*lac I*).

The M13 mp18-G1, -G2, -G7, and -G9 clones, containing SEQ ID NOs. 20-23, respectively, are digested with *EcoR* I and ligated into *EcoR* I digested pIN-IIIompA2 as described for M13 mp18-G4 above in this example. The resulting
20 plasmids, labeled pOMPAG1, pOMPAG2, pOMPAG7, pOMPAG9, are screened, expressed, purified, and characterized as described for the plasmid pOMPAG4.

INV1 α competent cells were transformed with pOMPAG4 and cultures containing the desired plasmid structure were grown further in order to obtain a large preparation of isolated pOMPAG4 plasmid using methods described herein.

25

E. Saporin expression in *E. coli*

The pOMPAG4 transformed *E. coli* cells were grown under conditions in which the expression of the saporin-containing protein is repressed by the lac repressor until the end of the log phase of growth, at which time IPTG was added to
30 induce expression of the saporin-encoding DNA.

To generate a large-batch culture of pOMPAG4 transformed *E. coli* cells, an overnight culture (approximately 16 hours growth) of JA221 *E. coli* cells transformed with the plasmid pOMPAG4 in LB broth (*see, e.g.,* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) containing 125 mg/ml ampicillin was diluted 1:100 into a flask containing 750 ml LB broth with 125 mg/ml ampicillin. Cells were grown at logarithmic phase with shaking at 37°C until the optical density at 550 nm reached 0.9 measured in a spectrophotometer.

In the second step, saporin expression was induced by the addition of IPTG (Sigma) to a final concentration of 0.2 mM. Induced cultures were grown for 2 additional hours and then harvested by centrifugation (25 min., 6500 x g). The cell pellet was resuspended in ice cold 1.0 M TRIS, pH 9.0, 2 mM EDTA (10 ml were added to each gram of pellet). The resuspended material was kept on ice for 20-60 minutes and then centrifuged (20 min., 6500 x g) to separate the periplasmic fraction of *E. coli*, which corresponds to the supernatant, from the intracellular fraction corresponding to the pellet.

The *E. coli* cells containing C-SAP construct in pET11a were grown in a high-cell density fed-batch fermentation with the temperature and pH controlled at 30°C and 6.9, respectively. A glycerol stock (1 ml) was grown in 50 ml Luria broth until the A_{600} reached 0.6. Inoculum (10 ml) was injected into a 7-l-Applikon (Foster City CA) fermentor containing 21 complex batch medium consisting of 5 g/l of glucose, 1.25 g/l each of yeast extract and tryptone (Difco Laboratories), 7 g/l of K_2HPO_4 , 8 g/l of KH_2PO_4 , 1.66 g/l of $(NH_4)_2SO_4$, 1 g/l of $MgSO_4 \cdot 7H_2O$, 2 ml/l of a trace metal solution (74 g/l of trisodium citrate, 27 g/l of $FeCl_3 \cdot 6H_2O$, 2.0 g/l of $CoCl_2 \cdot 6H_2O$, 2.0 g/l of $Na_2MoO_4 \cdot 2H_2O$, 1.9 g/l of $CuSO_4 \cdot 5H_2O$, 1.6 g/l of $MnCl_2 \cdot 4H_2O$, 1.4 g/l of $ZnCl_2 \cdot 4H_2O$, 1.0 g/l of $CaCl_2 \cdot 2H_2O$, 0.5 g/l of H_3BO_3), 2 ml/l of a vitamin solution (6 g/l of thiamin \cdot HCl, 3.05 g/l of niacin, 2.7 g/l of pantothenic acid, 0.7 g/l of pyridoxine \cdot HCl, 0.21 g/l of riboflavin, 0.03 g/l of biotin, 0.02 g/l of folic acid), and 100 mg/l of carbenicillin. The culture was grown for 12 h before initiating the continuous addition of a 40 \times solution of complex batch media lacking the phosphates

and containing only 25 ml/l, each, of trace metal and vitamin solutions. The feed addition continued until the A_{600} of the culture reached 85, at which time (approximately 9 h) the culture was induced with 0.1 mM isopropyl β -D-thiogalactopyranoside. During 4 h of post-induction incubation, the culture was fed with a solution containing 100 g/l of glucose, 100 g/l of yeast extract, and 200 g/l of tryptone. Finally, the cells were harvested by centrifugation (8000 \times g, 10 min) and frozen at -80°C until further processed.

The cell pellet (\approx 400 g wet mass) containing C-SAP was resuspended in 3 vol Buffer B (10 mM sodium phosphate pH 7.0, 5 mM EDTA, 5 mM EGTA, and 1 mM dithiothreitol). The suspension was passed through a microfluidizer three times at 124 Mpa on ice. The resultant lysate was diluted with NanoPure H₂O until conductivity fell below 2.7 mS/cm. All subsequent procedures were performed at room temperature.

The diluted lysate was loaded onto an expanded bed of Streamline SP cation-exchange resin (300 ml) equilibrated with buffer C (20 mM sodium phosphate pH 7.0, 1 mM EDTA) at 100 ml/min upwards flow. The resin was washed with buffer C until it appeared clear. The plunger was then lowered at 2 cm/min while washing continued at 70 ml/min. Upwards flow was stopped when the plunger was approximately 8 cm away from the bed and the plunger was allowed to move to within 0.5 cm of the packed bed. The resin was further washed at 70 ml/min downwards flow until A_{280} reached baseline. Buffer C plus 0.25 M NaCl was then used to elute proteins containing C-SAP at the same flow rate.

The eluate was buffer exchanged into buffer D (50 mM sodium borate pH 8.5, 1 mM EDTA) using the Sartoclon Mini crossflow filtration system with a 10000 NMolecular Massco module (Sartorius). The sample was then applied to a column of Source 15S (30 ml) equilibrated with buffer D. A 10-column-volume linear gradient of 0-0.3 M NaCl in buffer D was used to elute C-SAP at 30 ml/min.

F. Assay for cytotoxic activity

The ribosome inactivating protein activity of recombinant saporin was compared to the ribosome inactivating protein activity of native SAP in an *in vitro* assay measuring cell-free protein synthesis in a nuclease-treated rabbit reticulocyte

lysate (Promega). Samples of immunoaffinity-purified saporin were diluted in PBS and 5 μ l of sample was added on ice to 35 μ l of rabbit reticulocyte lysate and 10 μ l of a reaction mixture containing 0.5 μ l of Brome Mosaic Virus RNA, 1 mM amino acid mixture minus leucine, 5 μ Ci of tritiated leucine and 3 μ l of water. Assay tubes were
5 incubated 1 hour in a 30°C water bath. The reaction was stopped by transferring the tubes to ice and adding 5 μ l of the assay mixture, in triplicate, to 75 μ l of 1 N sodium hydroxide, 2.5% hydrogen peroxide in the wells of a Millititer HA 96-well filtration plate (Millipore). When the red color had bleached from the samples, 300 μ l of ice cold 25% trichloroacetic acid (TCA) were added to each well and the plate left on ice for
10 another 30 min. Vacuum filtration was performed with a Millipore vacuum holder. The wells were washed three times with 300 μ l of ice cold 8% TCA. After drying, the filter paper circles were punched out of the 96-well plate and counted by liquid scintillation techniques.

The IC₅₀ for the recombinant and native saporin were approximately 20
15 pM. Therefore, recombinant saporin-containing protein has full protein synthesis inhibition activity when compared to native saporin.

EXAMPLE 2

20

PREPARATION OF FGF MUTEINS

A. Materials and Methods

1. Reagents

Restriction and modification enzymes were purchased from BRL
25 (Gaithersburg, MD), Stratagene (La Jolla, CA) and New England Biolabs (Beverly, MA).

Plasmid pFC80, containing the basic FGF coding sequence, was a gift of Drs. Paolo Sarmientos and Antonella Isacchi of Farmitalia Carlo Erba (Milan, Italy). Plasmid pFC80, has been described in the PCT Application Serial No. WO 90/02800
30 and PCT Application Serial No. PCT/US93/05702, which are herein incorporated in

their entirety by reference. The sequence of DNA encoding bFGF in pFC80 is that set forth in PCT Application Serial No. PCT/US93/05702 and in SEQ ID NO. 52.

Plasmid isolation, production of competent cells, transformation and M13 manipulations were carried out according to published procedures (Sambrook et al., *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Purification of DNA fragments was achieved using the GeneClean II kit, purchased from Bio 101 (La Jolla, CA). Sequencing of the different constructions was performed using the Sequenase kit (version 2.0) of USB (Cleveland, OH).

10

2. Sodium dodecyl sulphate (SDS) gel electrophoresis and Western blotting

SDS gel electrophoresis was performed on a PhastSystem utilizing 20% gels (Pharmacia). Western blotting was accomplished by transfer of electrophoresed protein to nitrocellulose using the PhastTransfer system (Pharmacia), as described by the manufacturer. The antisera to SAP and basic FGF were used at a dilution of 1:1000. Horseradish peroxidase labeled anti-IgG was used as the second antibody as described (Davis, L., Dibner et al. (1986) *Basic Methods in Molecular Biology*, p. 1, Elsevier Science Publishing Co., New York).

20 B. Preparation of the mutagenized FGF by site-directed mutagenesis

Cysteine to serine substitutions were made by oligonucleotide-directed mutagenesis using the Amersham (Arlington Heights, IL) *in vitro*-mutagenesis system 2.1. Oligonucleotides encoding the new amino acid were synthesized using a 380B automatic DNA synthesizer (Applied Biosystems, Foster City, CA).

25

1. Mutagenesis

The oligonucleotide used for *in vitro* mutagenesis of cysteine 78 was AGGAGTGTCTGCTAACC (SEQ ID NO. 56), which spans nucleotides 225-241 of SEQ ID NO. 52). The oligonucleotide for mutagenesis of cysteine 96 was TTCTAAATCGGTTACCGATGACTG (SEQ ID NO. 57), which spans nucleotides 279-302 of SEQ ID NO. 52). The mutated replicative form DNA was transformed into

30

E. coli strain JM109 and single plaques were picked and sequenced for verification of the mutation. The FGF mutated gene was then cut out of M13, ligated into the expression vector pFC80, which had the non-mutated form of the gene removed, and transformed into *E. coli* strain JM109. Single colonies were picked and the plasmids
5 sequenced to verify the mutation was present. Plasmids with correct mutation were then transformed into the *E. coli* strain FICE 2 and single colonies from these transformations were used to obtain the mutant basic FGFs. Approximately 20 mg protein per liter of fermentation broth was obtained.

10 2. Purification of mutagenized FGF

Cells were grown overnight in 20 ml of LB broth containing 100 µg/ml ampicillin. The next morning the cells were pelleted and transferred to 500 ml of M9 medium with 100 µg/ml ampicillin and grown for 7 hours. The cells were pelleted and resuspended in lysis solution (10 mM TRIS, pH 7.4, 150 mM NaCl, lysozyme, 10 µ
15 g/mL, aprotinin, 10 µg/mL, leupeptin, 10 µg/mL, pepstatin A, 10 µg/mL and 1 mM PMSF; 45-60 ml per 16 g of pellet) and incubated while stirring for 1 hour at room temperature. The solution was frozen and thawed three times and sonicated for 2.5 minutes. The suspension was centrifuged; the supernatant saved and the pellet resuspended in another volume of lysis solution without lysozyme, centrifuged again
20 and the supernatants pooled. Extract volumes (40 ml) were diluted to 50 ml with 10 mM TRIS, pH 7.4 (buffer A). Pools were loaded onto a 5 ml Hi-Trap heparin-Sepharose column (Pharmacia, Uppsala, Sweden) equilibrated in 150 mM sodium chloride in buffer A. The column was washed with 0.6 M sodium chloride and 1 M sodium chloride in buffer A and then eluted with 2 M sodium chloride in buffer A.
25 Peak fractions of the 2 M elution, as determined by optical density at 280 nm, were pooled and purity determined by gel electrophoresis. Yields were 10.5 mg of purified protein for the Cys⁷⁸ mutant and 10.9 mg for the Cys⁹⁶ mutant.

The biological activity of [C78S]FGF and [C96S]FGF was measured on adrenal capillary endothelial cells in culture. Cells were plated at 3,000 per well in a 24
30 well plate in 1 ml of 10% calf serum-HDMEM. Cells were allowed to attach, and samples were added in triplicate at the indicated concentration and incubated for 48 h at

37°C. An equal quantity of samples was added and further incubated for 48 h. Medium was aspirated; cells were treated with trypsin (1 ml volume) to remove cells to 9 ml of Hemataill diluent and counted in a Coulter Counter. The results show that the two mutants that retain virtually complete proliferative activity of native basic FGF as judged by the ability to stimulate endothelial cell proliferation in culture.

EXAMPLE 3

PREPARATION OF MONO-DERIVATIZED NUCLEIC ACID

10

BINDING DOMAIN (MYOD)

MyoD at a concentration of 4.1 mg/ml is dialyzed against 0.1 M sodium phosphate, 0.1 M sodium chloride, pH 7.5. A 1.1 molar excess (563 µg in 156 µl of anhydrous ethanol) of SPDP (Pharmacia, Uppsala, Sweden) is added and the reaction mixture immediately agitated and put on a rocker platform for 30 minutes. The solution is then dialyzed against the same buffer. An aliquot of the dialyzed solution is examined for extent of derivatization according to the Pharmacia instruction sheet. The extent of derivatization is typically 0.79 to 0.86 moles of SPDP per mole of nucleic acid binding domain.

Derivatized myoD (32.3 mg) is dialyzed in 0.1 M sodium borate, pH 9.0 and applied to a Mono S 16/10 column equilibrated with 25 mM sodium chloride in dialysis buffer. A gradient of 25 mM to 125 mM sodium chloride in dialysis buffer elutes free and derivatized nucleic acid binding domain. The flow rate is 4.0 ml/min, 4 ml fractions are collected. Aliquots of fractions were assayed for protein concentration (BCA Protein Assay, Pierce Chemical, Chicago, IL) and for pyridylthione released by reducing agent. Individual fractions (25 to 37) are analyzed for protein concentration and pyridyl-disulfide concentration. The data indicate a separation according to the level of derivatization by SPDP. The initial eluting peak is composed of myoD that is approximately di-derivatized; the second peak is mono-derivatized and the third peak shows no derivatization. The di-derivatized material accounts for

approximately 20% of the three peaks; the second accounts for approximately 48% and the third peak contains approximately 32%. Material from the second peak is pooled and gives an average ratio of pyridyl-disulfide to myoD of 0.95. Fraction 33, which showed a divergent ratio of pyridine-2-thione to protein, was excluded from the pool.

- 5 Fractions that showed a ratio of SPDP to myoD greater than 0.85 but less than 1.05 are pooled, dialyzed against 0.1 M sodium chloride, 0.1 M sodium phosphate, pH 7.5 and used for derivatization with basic FGF.

10

EXAMPLE 4

PREPARATION OF MODIFIED NUCLEIC ACID BINDING DOMAIN (MYOD)

As an alternative to derivatization, myoD is modified by addition of a cysteine residue at or near the N-terminus-encoding portion of the DNA. The resulting myoD can then react with an available cysteine on an FGF or react with a linker or a linker attached to an FGF to produce conjugates that are linked via the added Cys.

Modified myoD is prepared by modifying DNA encoding the myoD (GenBank Accession No. X56677). DNA encoding Cys is inserted at position -1 or at a codon within 10 or fewer residues of the N-terminus. The resulting DNA is inserted into pET11a and pET15b and expressed in BL21 cells (NOVAGEN, Madison, WI).

A. Preparation of myoD with an added cysteine residue at the N-terminus

Primer #1 corresponding to the sense strand of myoD, nucleotides 121-144, incorporates a *NdeI* site and adds a Cys codon 5' to the start site for the mature protein

5'-CATATGTGTGAGCTACTGTCGCCACCGCTC-3' (SEQ ID NO. 58)

Primer #2 is an antisense primer complementing the coding sequence of nucleic acid binding domain spanning nucleotides 1054-1077 and contains a *BamHI* site.

5'-GGATCCGAGCACCTGGTATATCGGTGGGGG-3' (SEQ ID NO. 59)

MyoD DNA is amplified by PCR as follows using the above primers. A
5 clone containing a full-length DNA (or cDNA) for myoD (1 µl) is mixed in a final
volume of 100 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin,
2 mM MgCl₂, 0.2 mM dNTPs, 0.8 µg of each primer. Next, 2.5 U TaqI DNA
polymerase (Boehringer Mannheim) is added and the mixture is overlaid with 30 µl of
mineral oil (Sigma). Incubations are done in a DNA Thermal Cycler. Cycles include a
10 denaturation step (94°C for 1 min), an annealing step (60°C for 2 min), and an
elongation step (72°C for 3 min). After 35 cycles, a 10 µl aliquot of each reaction is run
on a 1.5% agarose gel to verify the correct structure of the amplified product.

The amplified DNA is gel purified and digested with *NdeI* and *BamHI*
and subcloned into *NdeI* and *BamHI*-digested plasmid containing FGF/myoD. This
15 digestion and subcloning step removes the FGF-encoding DNA and 5' portion of SAP
up to the *BamHI* site at nucleotides 555-560 (SEQ ID NO. 52) and replaces this portion
with DNA encoding a myoD molecule that contains a cysteine residue at position -1
relative to the start site of the native mature SAP protein.

20 B. Preparation of nucleic acid binding domain with a cysteine residue at position 4
or 10 of the native protein

These constructs are designed to introduce a cysteine residue at position
4 or 10 of the native protein by replacing the Ser residue at position 4 or the Val residue
at position 10 with cysteine.

25 MyoD is amplified by polymerase chain reaction (PCR) from the
parental plasmid encoding the FGF-nucleic acid binding domain fusion protein using
primers that incorporate a TGT or TGC codon at position 4 or 10.

The PCR conditions are performed as described above, using the
following cycles: denaturation step 94°C for 1 minute, annealing for 2 minutes at 60°C,
30 and extension for 2 minutes at 72°C for 35 cycles. The amplified DNA is gel purified,
digested with *NdeI* and *BamHI*, and subcloned into *NdeI* and *BamHI* digested pET11a.

This digestion removes the FGF and 5' portion of nucleic acid binding domain (up to the newly added *Bam*HI) from the parental FGF- myoD vector and replaces this portion with a myoD molecule containing a Cys at position 4 or 10 relative to the start site of the native protein.

- 5 The resulting plasmid is digested with *Nde*I/*Bam*HI and inserted into pET15b (NOVAGEN, Madison, WI), which has a His-TagTM leader sequence (SEQ ID NO. 60), that has also been digested *Nde*I/*Bam*HI.

DNA encoding unmodified myoD can be similarly inserted into a pET5b or pET11A and expressed as described below for the modified SAP-encoding DNA.

10

C. Expression of the modified nucleic acid binding domain-encoding DNA

- BL21(DE3) cells are transformed with the resulting plasmids and cultured as described in Example 2, except that all incubations were conducted at 30°C instead of 37°C. Briefly, a single colony is grown in LB AMP₁₀₀ to and OD₆₀₀ of 1.0-1.5 and then induced with IPTG (final concentration 0.1 mM) for 2 h. The bacteria are spun down.

D. Purification of modified nucleic acid binding domain

- Lysis buffer (20 mM NaPO₄, pH 7.0, 5 mM EDTA, 5 mM EGTA, 1 mM DTT, 0.5 µg/ml leupeptin, 1 µg/ml aprotinin, 0.7 µg/ml pepstatin) was added to the myoD cell paste (produced from pZ50B1 in BL21 cells, as described above) in a ratio of 1.5 ml buffer/g cells. This mixture is evenly suspended via a Polytron homogenizer and passed through a microfluidizer twice.

- The resulting lysate is centrifuged at 50,000 rpm for 45 min. The supernatant is diluted with SP Buffer A (20 mM NaPO₄, 1 mM EDTA, pH 7.0) so that the conductivity is below 2.5 mS/cm. The diluted lysate supernatant is then loaded onto a SP-Sepharose column, and a linear gradient of 0 to 30% SP Buffer B (1 M NaCl, 20 mM NaPO₄, 1 mM EDTA, pH 7.0) in SP Buffer A with a total of 6 column volumes is applied. Fractions containing myoD are combined and the resulting nucleic acid binding domain had a purity of greater than 90%. A buffer exchange step is used to get the SP eluate into a buffer containing 50 mM NaBO₃, 1 mM EDTA, pH 8.5 (S Buffer A). This sample is then applied to a Resource S column (Pharmacia, Sweden) pre-

equilibrated with S Buffer A. Pure nucleic acid binding domain is eluted off the column by 10 column volumes of a linear gradient of 0 to 300 mM NaCl in SP Buffer A.

In this preparation, ultracentrifugation is used clarify the lysate; other methods, such as filtration and using flocculents also can be used. In addition, Streamline S (PHARMACIA, Sweden) may also be used for large scale preparations.

EXAMPLE 5

PREPARATION OF CONJUGATES CONTAINING FGF MUTEINS

A. Coupling of FGF muteins to nucleic acid binding domain

1. Chemical Synthesis of [C78S]FGF-nucleic acid binding domain (CCFN2) and [C96S]FGF-nucleic acid binding domain (CCFN3)

[C78S]FGF or [C96S]FGF (1 mg; 56 nmol) that had been dialyzed against phosphate-buffered saline is added to 2.5 mg mono-derivatized nucleic acid binding domain (a 1.5 molar excess over the basic FGF mutants) and left on a rocker platform overnight. The next morning the ultraviolet-visible wavelength spectrum is taken to determine the extent of reaction by the release of pyridylthione, which adsorbs at 343 nm with a known extinction coefficient. The ratio of pyridylthione to basic FGF mutant for [C78S]FGF is 1.05 and for [C96S]FGF is 0.92. The reaction mixtures are treated identically for purification in the following manner: reaction mixture is passed over a HiTrap heparin-Sepharose column (1 ml) equilibrated with 0.15 M sodium chloride in buffer A at a flow rate of 0.5 ml/min. The column is washed with 0.6 M NaCl and 1.0 M NaCl in buffer A and the product eluted with 2.0 M NaCl in buffer A. Fractions (0.5 ml) are analyzed by gel electrophoresis and absorbance at 280 nm. Peak tubes are pooled and dialyzed versus 10 mM sodium phosphate, pH 7.5 and applied to a Mono-S 5/5 column equilibrated with the same buffer. A 10 ml gradient between 0 and 1.0 M sodium chloride in equilibration buffer is used to elute the product. Purity is determined by gel electrophoresis and peak fractions were pooled.

Under these conditions, virtually 100% of the mutant FGFs reacts with mono-derivatized myoD. Because the free surface cysteine of each mutant acts as a free sulfhydryl, it is unnecessary to reduce cysteines after purification from the bacteria. The resulting product is purified by heparin-Sepharose (data not shown), thus establishing that heparin binding activity of the conjugate is retained.

2. Expression of the recombinant FGFC78/96S-nucleic acid binding domain fusion proteins (FPFN4)

A two-stage method is used to produce recombinant FGF[C78/96S]-myoD protein (hereinafter FPFN4). Two hundred and fifty ml of LB medium containing ampicillin (100 µg/ml) are inoculated with a fresh glycerol stock of bacteria containing the plasmid. Cells are grown at 30°C in an incubator shaker to an OD₆₀₀ of 0.7 and stored overnight at 4°C. The following day, cells are pelleted and resuspended in fresh LB medium (no ampicillin). The cells are divided into 5 1-liter batches and grown at 30°C in an incubator shaker to an OD₆₀₀ of 1.5. IPTG is added to a final concentration of 0.1 mM and growth is continued for about 2 to 2.5 hours at which time cells were harvested by centrifugation.

20

EXAMPLE 6

RECOMBINANT PRODUCTION OF FGF-NUCLEIC ACID BINDING DOMAIN FUSION PROTEIN

A. General Descriptions

25 1. Bacterial Strains and Plasmids

E. coli strains BL21(DE3), BL21(DE3)pLysS, HMS174(DE3) and HMS174(DE3)pLysS were purchased from NOVAGEN, Madison, WI. Plasmid pFC80, described below, has been described in the WIPO International Patent Application No. WO 90/02800, except that the bFGF coding sequence in the plasmid designated pFC80 herein has the sequence set forth as SEQ ID NO. 52, nucleotides 1-465. The

30

plasmids described herein may be prepared using pFC80 as a starting material or, alternatively, by starting with a fragment containing the cII ribosome binding site (SEQ ID NO. 61) linked to the FGF-encoding DNA (SEQ ID NO. 52).

E. coli strain JA221 (lpp⁻ hdsM⁺ trpE5 leuB6 lacY recA1 F'[lacI^q lac⁺ pro⁺]) is publicly available from the American Type Culture Collection (ATCC), Rockville, MD 20852, under the accession number ATCC 33875. (JA221 is also available from the Northern Regional Research Center (NRRL), Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, under the accession number NRRL B-15211; *see also* U.S. Patent No. 4,757,013 to Inouye; and Nakamura et al., *Cell* 18:1109-1117, 1979). Strain INV1 α is commercially available from Invitrogen, San Diego, CA.

B. Construction of plasmids encoding FGF/nucleic acid binding domain fusion proteins

1. Construction of FGFM13 that contains DNA encoding the cI ribosome binding site linked to FGF

A Nco I restriction site is introduced into the nucleic acid binding domain-encoding DNA by site-directed mutagenesis using the Amersham *in vitro*-mutagenesis system 2.1. The oligonucleotide employed to create the Nco I restriction site is synthesized using a 380B automatic DNA synthesizer (Applied Biosystems). This oligonucleotide containing the Nco I site replaces the original nucleic acid binding domain-containing coding sequence.

In order to produce a bFGF coding sequence in which the stop codon was removed, the FGF-encoding DNA is subcloned into a M13 phage and subjected to site-directed mutagenesis. Plasmid pFC80 is a derivative of pDS20 (*see, e.g.*, Duester et al., *Cell* 30:855-864, 1982; *see also* U.S. Patent Nos. 4,914,027, 5,037,744, 5,100,784, and 5,187,261; *see also* PCT International Application No. WO 90/02800; and European Patent Application No. EP 267703 A1), which is almost the same as plasmid pKG1800 (*see* Bernardi et al., *DNA Sequence* 1:147-150, 1990; *see also* McKenney et al. (1981) pp. 383-415 in *Gene Amplification and Analysis 2: Analysis of Nucleic Acids by Enzymatic Methods*, Chirikjian et al. (eds.), North Holland Publishing

Company, Amsterdam) except that it contains an extra 440 bp at the distal end of *galK* between nucleotides 2440 and 2880 in pDS20. Plasmid pKG1800 includes the 2880 bp *EcoR* I-*Pvu* II of pBR322 that contains the contains the ampicillin resistance gene and an origin of replication.

- 5 Plasmid pFC80 is prepared from pDS20 by replacing the entire *galK* gene with the FGF-encoding DNA of SEQ ID NO. 52, inserting the trp promoter (SEQ ID NO. 62) and the bacteriophage lambda cII ribosome binding site (SEQ. ID No. 61; see, e.g., Schwarz et al., *Nature* 272:410, 1978) upstream of and operatively linked to the FGF-encoding DNA. The Trp promoter can be obtained from plasmid pDR720
10 (Pharmacia PL Biochemicals) or synthesized according to SEQ ID NO. 62. Plasmid pFC80, contains the 2880 bp *EcoR* I-*Bam*H I fragment of plasmid pSD20, a synthetic *Sal* I-*Nde* I fragment that encodes the Trp promoter region:

EcoR I

- 15 AATTCCCCTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAGCTTGGCTGCAG

and the cII ribosome binding site (SEQ ID NO. 61)):

Sal I

Nde I

- 20 GTCGACCAAGCTTGGGCATACATTCAATCAATTGTTATCTAAGGAAATACTTACATATG

- The FGF-encoding DNA is removed from pFC80 by treating it as follows. The pFC80 plasmid was digested by *Hga* I and *Sal* I, which produces a fragment containing the CII ribosome binding site linked to the FGF-encoding DNA.
25 The resulting fragment is blunt ended with Klenow's reagent and inserted into M13mp18 that has been opened by *Sma* I and treated with alkaline phosphatase for blunt-end ligation. In order to remove the stop codon, an insert in the ORI minus direction is mutagenized using the Amersham kit, as described above, using the following oligonucleotide (SEQ ID NO. 63): GCTAAGAGCGCCATGGAGA, which
30 contains one nucleotide between the FGF carboxy terminal serine codon and a *Nco* I

restriction site; it replaces the following wild type FGF encoding DNA having SEQ ID NO. 64:

5 GCT AAG AGC TGA CCA TGG AGA
Ala Lys Ser STOP Pro Trp Arg

The resulting mutant derivative of M13mp18, lacking a native stop codon after the carboxy terminal serine codon of bFGF, was designated FGFM13. The mutagenized region of FGFM13 contained the correct sequence (SEQ ID NO. 65).

10

2. Preparation of a plasmid that encodes the FGF/MyoD fusion protein

Plasmid FGFM13 is cut with *Nco* I and *Sac* I to yield a fragment containing the CII ribosome binding site linked to the bFGF coding sequence with the stop codon replaced.

15 An M13mp18 derivative containing the myoD coding sequence is also cut with restriction endonucleases *Nco* I and *Sac* I, and the bFGF coding fragment from FGFM13 was inserted by ligation to DNA encoding the fusion protein bFGF- myoD into the M13mp18 derivative to produce mpFGF- myoD, which contains the CII ribosome binding site linked to the FGF-nucleic acid binding domain fusion gene.

20 Plasmid mpFGF- myoD is digested with *Xba* I and *Eco*R I and the resulting fragment containing the bFGF- myoD coding sequence is isolated and ligated into plasmid pET-11a (available from NOVAGEN, Madison, WI; for a description of the plasmids see U.S. Patent No. 4,952,496; see also Studier et al., *Meth. Enz.* 185:60-89, 1990; Studier et al., *J. Mol. Biol.* 189:113-130, 1986; Rosenberg et al., *Gene* 25 56:125-135, 1987) that has also been treated with *Eco*R I and *Xba* I.

E. coli strain BL21(DE3)pLysS (NOVAGEN, Madison WI) may be transformed with the plasmid containing the fusion gene.

Plasmid FGF/myoD may be digested with *Eco*R I, the ends repaired by adding nucleoside triphosphates and Klenow DNA polymerase, and then digested with 30 *Nde* I to release the FGF-encoding DNA without the CII ribosome binding site. This fragment is ligated into pET 11a, which is *Bam*H I digested, treated to repair the ends,

and digested with *Nde* I. The resulting plasmid includes the T7 transcription terminator and the pET-11a ribosome binding site.

Plasmid FGF/myoD may be digested with *EcoR* I and *Nde* I to release the FGF-encoding DNA without the CII ribosome binding site and ends are repaired as
5 described above. This fragment may be ligated into pET 12a, which had been *Bam*H I digested and treated to repair the ends. The resulting plasmid includes DNA encoding the OMP T secretion signal operatively linked to DNA encoding the fusion protein.

3. Preparation of a plasmid that encodes FGF2-protamine fusion protein

Protamines are small basic DNA binding proteins, approximately 6.8 kD
10 in molecular weight with a isoelectric point of 12.175. Twenty-four of the fifty one amino acids are strongly basic. Human protamine has been shown to condense genomic DNA for packaging into the sperm head. The positive charges of the protamine react with the negative charges of the phosphate backbone of the DNA.

A FGF-protamine fusion protein that has the ability to bind to the FGF
15 receptor and bind DNA with high affinity is constructed for expression in *E. coli*. The sequence for the human protamine gene is obtained from GenBank (accession no. Y00443). Four overlapping oligonucleotides (60mers) are generated and used to amplify the protamine gene. The amplified product is purified and ligated into the bacterial expression vector pET11a (Novagen). To facilitate subcloning, a *Nco*I and
20 *Bam*HI site are incorporated into the primers. The fragment is synthesized by annealing the 4 oligos (2 sense and 2 antisense) with 20 base overlaps and using PCR to fill-in and amplify the fragments. The PCR products are digested with *Nco*I and *Bam*HI, and subcloned into pBluescript SK+. The insert sequence is verified. The sequenced product is then cloned downstream and in-frame with FGF2, which has been previously
25 cloned into the pET11a expression plasmid. The oligos used to generate fragment A are (5'-3'):

PT1:

TACATGCCATGGCCAGGTACAGATGCTGTCTCGCAGCCAGAGCCGGAGCAGAT
30 ATTACCGCC (SEQ ID NO.: 85)

PT2:

GCAGCTCCGCCTCCTTCGTCTGCGACTTCTTTGTCTCTGGCGGTAATATCTGC
TCCGGCT (SEQ ID NO.: 86)

5

PT3:

GACGAAGGAGGCGGAGCTGCCAGACACGGAGGAGAGCCATGAGGTGCTGC
CGCCCCAGGT (SEQ ID NO.: 87)

10 PT4:

ATATATCCTAGGTTAGTGTCTTCTACATCTCGGTCTGTACCTGGGGCGGCAG
CACCTCA (SEQ ID NO.: 88)

Competent bacterial cells, BL21 (DE3), are transformed with the pET11-
15 FGF2-protamine construct. The cells are initially plated on LB agar plates containing
100 µg/ml ampicillin. A glycerol stock made from an individual colony added to 1 ml
fresh LB broth and then to 250 ml of LB broth. The cells are grown to an OD₆₀₀ of 0.7
and induced with IPTG. The culture is harvested 4 hours after induction. The
suspension is centrifuged; the supernatant is saved and the pellet is resuspended in lysis
20 buffer, centrifuged again and the supernatants pooled. A sample of the pellet and the
supernatant are analyzed by Western analysis using antibodies to FGF2 to determine the
percentage of fusion protein within each fraction. Soluble protein is purified. Briefly,
the cells are pelleted and resuspended in buffer A (10 mM sodium phosphate, pH 6.0,
containing 10 mM EDTA, 10 mM EGTA and 50 mM NaCl) and passed through a
25 microfluidizer (Microfluidics Corp., Newton, MA) to break open the bacteria and shear
DNA. The resultant mixture is diluted and loaded onto an expanded bed Streamline SP
cation-exchange resin. The column is washed with step gradients of increasing
concentrations of NaCl. The eluted material is analyzed by Western analysis for
fractions containing the fusion protein. These fractions are pooled, diluted, and loaded
30 onto a Heparin-Sepharose affinity column. After washing, the bound proteins are eluted

in a batch-wise manner in buffer containing 1 M NaCl and then in buffer containing 2 M NaCl. Peak fractions of the 2M elution, as determined by optical density at 280 nm, are pooled and the purity determined by gel electrophoresis and Western analysis. The final pool of material will be loaded onto a column of Sephacryl S-100 equilibrated
5 with 20 mM HEPES pH 7.4, 150 mM NaCl.

Fusion protein located in the pellet is isolated, solubilized and refolded. Briefly, each culture pellet is thawed completely and resuspended in buffer A (10 mM Tris, 1 mM EDTA, pH 8.0 + 0.1 mg/ml lysozyme). The mixture is sonicated on ice, centrifuged at 16,000 X g, and the supernatant discarded. Inclusion bodies are
10 solubilized with solubilization buffer: (6 M guanidine-HCl, 100 mM Tris, 150 mM NaCl, 50 mM EDTA, 50 mM EGTA, pH 9.5), vortexed, incubated for 30 minutes at room temperature, and centrifuged at 35,000 X g for 15 minutes. The supernatant is saved and diluted 1:10 in dilution buffer (100 mM Tris, 10 mM EDTA, 1% monothioglycerol, 0.25 M L-arginine, pH 9.5). The material is stirred, covered, at 4°C
15 for 2 hours and then centrifuged at 35,000 X g for 20 minutes. The supernatant is dialyzed in against 5 liters PBS, pH 8.8, for 24 hours at 4°C with 3 changes of fresh PBS. The material is concentrated approximately 10-fold using size-exclusion spin columns. The soluble refolded material is then analyzed by gel electrophoresis.

Expression of the FGF-protamine fusion protein can be achieved in
20 mammalian cells by excising the insert with restriction enzymes *NdeI* and *BamHI* and ligating into a mammalian expression vector.

C. Expression of the recombinant bFGF-nucleic acid binding domain fusion proteins

25 A two-stage method is used to produce recombinant bFGF-myoD protein (hereinafter bFGF-nucleic acid binding domain fusion protein).

Three liters of LB broth containing ampicillin (50 µg/ml) and chloramphenicol (25 µg/ml) are inoculated with pFS92 plasmid-containing bacterial cells (strain BL21(DE3)pLysS) from an overnight culture (1:100 dilution). Cells are
30 grown at 37°C in an incubator shaker to an OD₆₀₀ of 0.7. IPTG (Sigma Chemical,

St. Louis, MO) is added to a final concentration of 0.2 mM and growth was continued for 1.5 hours at which time cells were centrifuged.

Experiments have shown that growing BL21(DE3)pLysS cells at 30°C instead of 37°C improves yields. Thus, cells are grown at 30°C to an OD₆₀₀ of 1.5 prior to induction. Following induction, growth is continued for about 2 to 2.5 hours at which time the cells are harvested by centrifugation.

The pellet is resuspended in lysis solution (45-60 ml per 16 g of pellet; 20 mM TRIS, pH 7.4, 5 mM EDTA, 10% sucrose, 150 mM NaCl, lysozyme, 100 µg/ml, aprotinin, 10 µg/ml, leupeptin, 10 µg/ml, pepstatin A, 10 µg/ml and 1 mM PMSF) and incubated with stirring for 1 hour at room temperature. The solution is frozen and thawed three times and sonicated for 2.5 minutes. The suspension is centrifuged at 12,000 X g for 1 hour; the resulting first-supernatant saved and the pellet is resuspended in another volume of lysis solution without lysozyme. The resuspended material is centrifuged again to produce a second-supernatant, and the two supernatants are pooled and dialyzed against borate buffered saline, pH 8.3.

D. Affinity purification of bFGF-nucleic acid binding domain fusion protein

Thirty ml of the dialyzed solution containing the bFGF-nucleic acid binding domain fusion protein from Example 5.C. is applied to HiTrap heparin-Sepharose column (Pharmacia, Uppsala, Sweden) equilibrated with 0.15 M NaCl in 10 mM TRIS, pH 7.4 (buffer A). The column is washed first with equilibration buffer; second with 0.6 M NaCl in buffer A; third with 1.0 M NaCl in buffer A; and finally eluted with 2 M NaCl in buffer A into 1.0 ml fractions. Samples were assayed by the ELISA method.

bFGF-nucleic acid binding domain fusion protein elutes from the heparin-Sepharose column at the same concentration (2 M NaCl) as native and recombinantly-produced bFGF, indicating that the heparin affinity is retained in the bFGF-SAP fusion protein.

E. Characterization of the bFGF-nucleic acid binding domain fusion protein by Western blot

SDS gel electrophoresis is performed on a Phastsystem utilizing 20% acrylamide gels (Pharmacia). Western blotting is accomplished by transfer of the electrophoresed protein to nitrocellulose using the PhastTransfer system (Pharmacia), as described by the manufacturer. Antisera to bFGF is used at a dilution of 1:1000. Horseradish peroxidase labeled anti-IgG is used as the second antibody (Davis et al., *Basic Methods in Molecular Biology*, New York, Elsevier Science Publishing Co., pp 1-338, 1986).

Anti-FGF antisera should bind to a protein with an approximate molecular weight of 53,000, which corresponds to the sum of the independent molecular weights of nucleic acid binding domain (35,000) and bFGF (18,000).

15

EXAMPLE 7

PREPARATION OF FGF-NUCLEIC ACID BINDING DOMAIN CONJUGATES THAT CONTAIN LINKERS ENCODING PROTEASE SUBSTRATES

A. Synthesis of oligos encoding protease substrates

Complementary single-stranded oligos in which the sense strand encodes a protease substrate, have been synthesized either using a cyclone machine (Millipore, MA) according the instructions provided by the manufacturer, or were made by Midland Certified Reagent Co. (Midland, TX) or by National Biosciences, Inc. (MN). The following oligos have been synthesized.

25

1. Cathepsin B substrate linker

5' - CCATGGCCCTGGCCCTGGCCCTGGCCCTGGCCATGG SEQ ID NO: 66

2. Cathepsin D substrate linker

5' - CCATGGGCGGATCGGGCTTCCTGGGCTTCGGCTTCCTGG
GCTTCGCCAT GG -3' SEQ ID NO: 67

30

3. Trypsin substrate linker

5' - CCATGGGCGGATCGGGCGGTGGGTGCGCTGGTAATAGAGT

CAGAAGATCAGTCGGAAGCAGCCTGTCTTGCGGTGGTCTC

GACCTGCAGG CCATGG-3' SEQ ID NO: 68

4. Gly₄Ser

5'- CCATGGGCGG CGGCGGCTCT GCCATGG -3' SEQ ID NO: 47

5. (Gly₄Ser)₂

5'- CCATGGGCGGCGGCGGCTCTGGCGGCGGCGGCTC
TGCCATGG -3' SEQ ID NO: 48

6. (Ser₄Gly)₄

5'- CCATGGCCTCGTCGTCGTCGGGCTCGTCGTCGTC
GGGCTCGTCGTCGTCGGGCTCGTCGTCGTCGGGC
GCCATGG -3' SEQ ID NO: 49

7. (Ser₄Gly)₂

5'- CCATGGCCTCGTCGTCGTCGGGCTCGTCGTCGTC
GGGCGCCATGG -3' SEQ ID NO: 50

8. Thrombin substrate linker

CTG GTG CCG CGC GGC AGC SEQ ID NO. 69
Leu Val Pro Arg Gly Ser

9. Enterokinase substrate linker

GAC GAC GAC GAC CCA SEQ ID NO. 70
20 Asp Asp Asp Asp Lys

10. Factor Xa substrate

ATC GAA GGT CGT SEQ ID NO. 71
Ile Glu Gly Arg

25 B. Preparation of DNA constructs encoding FGF-Linker-nucleic acid binding domain

The complementary oligos are annealed by heating at 95°C for 15 min., cooled to room temperature, and then incubated at 4°C for a minute to about an hour. Following incubation, the oligos are digested with *Nco*I and ligated overnight at a 3:1 (insert:vector) ratio at 15°C to *Nco*I-digested plasmid which has been treated with alkaline phosphatase (Boehringer Mannheim).

Bacteria (Novablue (NOVAGEN, Madison, WI)) are transformed with the ligation mixture (1 µl) and plated on LB-amp or LB-Kan, depending upon the plasmid). Colonies are selected, clones isolated and sequenced to determine orientation of the insert. Clones with correct orientation are used to transform strain expression

strain BL21(DE3) (NOVAGEN, Madison, WI). Glycerol stocks are generated from single transformed colonies. The transformed strains are cultured as described in Example 2 and fusion proteins with linkers were expressed.

The DNA and amino acid sequences of exemplary fusion proteins, containing cathepsin B substrate (FPFS9), cathepsin D substrate (FPFS5), Gly₄Ser (FPFS7), (Gly₄Ser)₂ (FPFS8), trypsin substrate (FPFS6), (Ser₄Gly)₄ (FPFS12) and (Ser₄Gly)₂ (FPFS11) linkers, respectively, are set forth in SEQ ID NOs. 72-78.

EXAMPLE 8

10 FGF-POLY-L-LYSINE (FGF2-K) COMPLEXED WITH A PLASMID ENCODING β -GALACTOSIDASE

A. Derivatization of poly-L-lysine

Polylysine polymer with average lengths of 13, 39, 89, 152, and 265
15 (K₁₃, K₃₉, K₈₄, K₁₅₂, K₂₆₅) are purchased from a commercial vendor (Sigma, St. Louis, MO) and dissolved in 0.1 M NaPO₄, 0.1 M NaCl, 1 mM EDTA, pH 7.5 (buffer A) at 3-5 mg/ml. Approximately 30 mg of poly-L-lysine solution is mixed with 0.187 ml of 3 mg/ml N-succinimidyl-3-(pyridyldithio)propionate (SPDP) in anhydrous ethanol resulting in a molar ratio of SPDP/poly-L-lysine of 1.5 and incubated at room
20 temperature for 30 minutes. The reaction mixture is then dialyzed against 4 liters of buffer A for 4 hours at room temperature.

B. Conjugation of derivatized polylysine to FGF2-3

A solution containing 28.5 mg of poly-L-lysine-SPDP is added to 12.9
25 mg of FGF2-3 ([C96S]-FGF2) in buffer A and incubated overnight at 4°C. The molar ratio of poly-L-lysine-SPDP/FGF2-3 is approximately 1.5. Following incubation, the conjugation reaction mixture is applied to a 6 ml Resource S (Pharmacia, Uppsala, Sweden) column. A gradient of 0.15 M to 2.1 M NaCl in 20 mM NaPO₄, 1 mM EDTA, pH 8.0 (Buffer B) over 24 column volumes is used for elution. The FGF2-
30 3/poly-L-lysine conjugate, called FGF2-K, is eluted off the column at approximately 1.8-2 M NaCl concentration. Unreacted FGF2-3 is eluted off by 0.5-0.6 M NaCl.

The fractions containing FGF2-K are concentrated and loaded onto a gel-filtration column (Sephacryl S100) for buffer exchange into 20 mM HEPES, 0.1 M NaCl, pH 7.3. The molecular weight of FGF-K152 as determined by size exclusion HPLC is approximately 42 kD. To determine if the conjugation procedure interferes with the ability of FGF2-3 to bind heparin, the chemical conjugate FGF2-K is loaded onto a heparin column and eluted off the column at 1.8- 2.0 M NaCl. In comparison, unconjugated FGF2-3 is eluted off heparin at 1.4 - 1.6 M NaCl. This suggests that poly-L-lysine contributes to FGF2-3 ability to bind heparin. The ability of poly-L-lysine 152 to bind heparin is not determined; poly-L-lysine 84 elutes at approximately 1.6 M NaCl. Histone HI-polylysine was purchased and cytochrome C was conjugated to polylysine as described herein.

A sample of FGF2-K is electrophoresed on SDS-PAGE under non-reducing and reducing conditions. The protein migrates at the same molecular weight as FGF. Under non-reducing conditions the conjugate does not enter the gel because of its high charge density (Figure 1, lanes 1, 2, non-reducing; lanes 3, 4, reducing).

A standard proliferation assay using aortic bovine endothelial cells is performed to determine if the conjugation procedure reduced the ability of FGF2-3 ability to stimulate mitogenesis. The results reveal that FGF2-K is equivalent to FGF2-3 in stimulating proliferation (Figure 2).

C. FGF2-3-poly-L-lysine-nucleic acid complex formation

Optimal conditions for complex formation are established. Varying quantities (0.2 to 200 μ g) of β -galactosidase encoding plasmid nucleic acid pSV β or pNASS- β (lacking a promoter) are slowly mixed with 100 μ g of FGF2-K in 20 mM HEPES pH 7.3, 0.15 M NaCl. The reaction is incubated for 1 hour at room temperature. Nucleic acid binding to the FGF-lysine conjugate is confirmed by gel mobility shift assay using 32 P-labeled SV40- β -gal nucleic acid cut with *HincII* restriction endonuclease. In brief, SV40 β -gal nucleic acid is digested with *HincII* restriction endonucleases; ends are labeled by T₄ PNK following dephosphorylation with calf intestinal alkaline phosphatase. To each sample of 35 ng of 32 P-labeled nucleic acid increasing amounts of FGF-polylysine conjugate is added to the mixture.

The protein/nucleic acid mixture is electrophoresed in an agarose gel with 1 X TAE buffer. Binding of the conjugate to the radiolabeled DNA is shown by a shift in the complex to the top of the well. (Figure 3.) As seen in Figure 3D, as little as 10 ng of K_{84} causes a complete shift of restriction fragments indicating binding. With K_{13} , 100
5 ng of poly-L-lysine was required (Figure 3C). With K_{265} , 10 ng was required (Figure 3E).

The optimal length of poly-L-lysine and weight ratios is determined by conjugation of FGF2-3 to poly-lysine of different lengths. DNA encoding β -galactosidase was complexed with the conjugates at 10:1, 5:1, 2:1, 1:1, and 0.5:1
10 (Figure 4, lanes 1-5, respectively) (w/w) ratios. The ability of these FGF2-K complexes to bind DNA was determined by measuring the ability of FGF to promote the uptake of plasmid DNA into cells. FGF2-K conjugates were evaluated at various protein to DNA ratios for their ability to deliver pSV β -gal DNA into cells (Figure 4).

Briefly, the complexes were incubated for 1 hr at room temperature and
15 then added to COS cells for 48 hrs. Cell extracts were prepared and assayed for β -gal enzyme activity. Briefly, cells are washed with 1 ml of PBS (Ca^{+2} and Mg^{+2} free) and lysed. The lysate was vortexed and cell debris removed by centrifugation. The lysate was assayed for β -gal activity as recommended by the manufacturer (Promega, Madison, WI). The β -gal activity was normalized to total protein. As seen in Figure 4,
20 lane 3, a 2:1 (w/w) ratio of FGF2-K:DNA gave maximal enzyme activity.

In addition, toroid formation, which correlates with increased gene expression, was assessed by electron microscopy. A representative toroid at a protein to DNA ratio of 2:1 is shown in Figure 5, upper panel. Toroidal structures are absent, or only partially formed, at low ratios (e.g., 0.5:1) (Figure 5, lower panel).

25 A proliferation assay is performed to determine if the condensed nucleic acid had an effect on the ability of FGF2-K to bind to cognate receptor and stimulate mitogenesis. The proliferation assay shows that only the highest dose of nucleic acid (200 μ g) has a slightly inhibitory effect on proliferation as compared to FGF2-3 plus poly-L-lysine + DNA (Figure 6).

A FGF2-K84-DNA at a protein:DNA ratio of 2:1 is introduced into COS cells and an endothelial cell line, ABAE, both of which express FGF receptors. The cells are subsequently assayed for β -galactosidase enzyme activity. COS and ABAE cells are grown on coverslips and incubated with the different ratios of FGF2-K:DNA
5 for 48 hours. The cells are then fixed and stained with X-gal. Maximal β -galactosidase enzyme activity is seen when 50 μ g of pSV β per 100 μ g of FGF2-3-polylysine conjugate is used.

FGF2-K84-pSV β -gal at a protein to DNA ratio of 2:1 was added to various cell lines and incubated for 48 hr. Cell extracts were prepared, assayed for
10 β -gal activity and total protein. As shown in Figure 7A, COS, B16, NIH3T3, and BHK cell lines were all able to take up complex and express β -gal.

The expression of β -gal requires FGF2 for targeting into cells. pSV β or pNASS β plasmid DNA was incubated with (Figure 7B, lanes 1, 2) or without (lanes 3, 4) FGF2-K84 for 1 hr at room temperature. Complexes were added to COS cells for 48
15 hr. Cell extracts were assayed for β -gal activity and normalized to total protein. Only background β -gal activity was seen unless the plasmid was complexed with FGF2/K84. Expression of β -gal is seen to be both time and dose-dependent (Figures 7C and 7D).

Sensitivity of the receptor mediated gene delivery system is determined using the optimized FGF2-K/DNA ratio for complex formation. Increasing amounts of
20 the FGF2-K/DNA complex is added to cells. 100 μ g of FGF2-K was mixed with 50 μ g of pSV β for 1 hour at room temperature. The COS and endothelial cells are incubated with increasing amounts of condensed material (0 ng, 1 ng, 10 ng, 100 ng, 1000 ng and 10,000 ng). The cells are incubated for 48 hours and then were assayed for β -galactosidase activity. In addition, cells grown on cover slips are treated with 1000
25 ng of FGF2-K-DNA for 48 hours, then fixed and stained using X-gal. The β -gal enzyme assay reveals that with increasing amounts of material there is an increase in enzyme activity. (Figure 7D) Cells incubated with X-gal show blue staining throughout the cytoplasm in approximately 3% of the cells on the coverslip.

Targeting of the complexes is specific for the FGF receptor. First, as
30 seen in Figure 8A, FGF2-K84-pSV β -gal resulted in enzyme activity (lane 1), while only

background levels of activity were seen with FGF2+K84+DNA (lane 2), FGF2+DNA (lane 3), K84+DNA (lane 4), DNA (lane 5), FGF2-K84 (lane 6), FGF2 alone (lane 7) and K84 alone (lane 8). The expression of β -gal is specifically inhibited if free FGF2 is added during transfection (Figure 8B). Moreover, the addition of heparin attenuates the expression of β -gal (Figure 8C). Moreover, histone HI and cytochrome C were ineffective in delivering pSV β -gal (Figure 8C).

Taken together, these findings support the hypothesis that the targeted DNA is introduced into receptor-bearing cells via the high affinity FGF receptor. Because histone can bind heparin sulfate yet fails to elicit a signal, the introduction of DNA appears independent of the low affinity FGF receptor or non-specific endocytosis.

D. Effect of endosome-disruptive peptides

Targeting is mediated by passage of the complex through endosomes. Chloroquine, which was added to complexes before transfection, resulted in an 8-fold increase in β -gal activity (Figure 9A).

Based on this, the effect of endosome disruptive peptides was evaluated. The peptide INF7, GLF EAIEGFIEN GWEGMIDGWYGC, derived from influenza virus, was synthesized. A complex between FGF2-K84 (5 μ g) and pSV β -gal plasmid DNA (5 μ g) was formed. At this ratio, approximately half of the negative charge of the DNA was neutralized by the conjugate. K84, poly-L-lysine, was further added to saturate binding to the remaining DNA. The INF7 peptide was added 30 minutes later. The complex is added to COS cells and β -gal activity is assayed 48 or 72 hr later.

The amount of free polylysine necessary to neutralize the DNA and allow INF7 to complex was determined. Polylysine was added at 4, 10, or 25 μ g to the FGF2-K84/pSV β -gal complex. To each of these complexes four different concentrations of INF7 were added. Maximal β -gal expression was seen with 4 μ g of K84 and 12 μ g of INF7 (Figure 13A). When higher amounts of poly-lysine were used, more cell death resulted. The optimal amount of INF7 was determined using 4 μ g of polylysine. As seen in Figure 13B, 24 μ g of INF7 gave maximal β -gal activity. At 72

hr, 48 μ g of INF7 gave maximal β -gal activity (approximately 20-32 fold enhancement) (Figure 13C).

When an endosome disruptive peptide was included in the complex, expression of β -gal was increased 26-fold (Figure 9B). Concomitant with this increased level of expression was an increase in the number of cells expressing β -gal. As seen in Figure 9C, when endosome disruptive peptide (EDP) was present (right panel), 1%-5% of cells express β -gal in comparison to 0.1%-0.3% without EDP added (left panel).

10

EXAMPLE 9

CYTOTOXIC ACTIVITY OF FGF/POLY-L-LYSINE

BOUND TO SAP DNA PLASMID

The cytotoxicity assay measures viable cells after transfection with a cytocide-encoding agent. When FGF-2 is the receptor-binding internalized ligand, COS7 cells, which express FGFR, may be used as targets, and T47D, which does not express a receptor for FGF-2 at detectable levels, may be used as negative control cells.

Cells are plated at 38,000 cells/well and 48,000 cells/well in a 12-well tissue culture plate in RPMI 1640 supplemented with 5% FBS. The complex FGF2-K/pZ200M (a plasmid which expresses saporin) is incubated with COS7 or T47D cells for 48 hrs. Controls include FGF2-K alone, pZ200M alone, and FGF-2 plus poly-L-lysine plus pZ200M. Following incubation, cells are rinsed in PBS lacking Mg^{++} and Ca^{++} . Trypsin at 0.1% is added for 10 min and cells are harvested and washed. Cell number from each well is determined by a Coulter particle counter (or equivalent method). A statistically significant decrease in cell number for cells incubated with FGF2-K/pZ200M compared to FGF2-K or pZ200M alone indicates sufficient cytotoxicity.

FGF2-polylysine-DNASAP complexes show selective cytotoxicity. To optimize the expression of the plant RIP, saporin, in mammalian cells, a synthetic saporin gene using preferred mammalian codons and introduced a "Kozak" sequence for

translation initiation. The synthetic gene was then cloned into SV40 promoter and promoterless expression vectors. Because the expression of SAP from SAP-encoding DNA would only be feasible if the mammalian ribosome can synthesize the protein (SAP) prior to its inactivation by the SAP synthesized, the enzymatic activity of saporin encoded by the synthetic gene was tested. SAP was cloned into a T7/SP6 promoter plasmid and sense RNA was generated using T7 RNA polymerase. The RNA was then added to a mammalian in vitro translation assay. The results from this cell-free in vitro translation assay clearly show that the saporin expressed in a mammalian system can inhibit the expression of protein mutagenesis (Figure 10). When added above to the lysate, SAP mRNA is translated into a protein that has the anticipated molecular weight of the saporin protein (lane 2). Similarly, when luciferase mRNA is added to the lysate, a molecule consistent with the luciferase protein is detected (lane 3). In contrast, if SAP mRNA is added to the lysate along with or 30 minutes prior to luciferase mRNA, saporin activity is detected (lanes 4 and 5).

Transfection of cells with SAP DNA demonstrates cytotoxicity. When a mammalian expression vector encoding saporin is transiently expressed in NIH 3T3 cells using CaPO₄, there is a >65% decrease in cell survival (lane 3) compared to cells mock transfected (lane 1) or transfected with DNA encoding β -gal (lane 2) (Figure 11).

To determine whether the FGF2-K can transfer plasmid DNA encoding SAP into FGF receptor bearing cells, FGF2-K was condensed with the pSV40-SAP plasmid DNA at a ratio of 2:1 (w:w). BHK 21 and NIH 3T3 cells were used as the target cells. The cells (24,000 cells/well) were incubated with either FGF2-K-DNASAP or an FGF2-K-DNA β -gal complex. After 72 hours of incubation, cell number was determined. As shown in Figure 12, there is a significant decrease in cell number when cells are incubated with the FGF2-K-DNASAP complex compared to cells incubated with the FGF2-K-DNA β -gal complex.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration,

various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

119

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Prizm Pharmaceuticals, Inc.

(ii) TITLE OF INVENTION: COMPOSITIONS CONTAINING NUCLEIC ACIDS AND LIGANDS FOR THERAPEUTIC TREATMENT

(iii) NUMBER OF SEQUENCES: 106

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SEED and BERRY
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 16-MAY-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Nottenburg Ph.D., Carol
(B) REGISTRATION NUMBER: 39.317
(C) REFERENCE/DOCKET NUMBER: 760100.415PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (206) 622-4900
(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 473 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 13..456
(D) OTHER INFORMATION: /product= "VEGF₁₂₁-encoding DNA"

120

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 13..90
- (D) OTHER INFORMATION: /product= leader-encoding sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCCGAAA CC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC CTT	48
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu	
1 5 10	
GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC	96
Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro	
15 20 25	
ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC ATG	144
Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met	
30 35 40	
GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG GAC	192
Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp	
45 50 55 60	
ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC	240
Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser	
65 70 75	
TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG	288
Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu	
80 85 90	
GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG CGG	336
Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg	
95 100 105	
ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA CAG	384
Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln	
110 115 120	
CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA GAA	432
His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu	
125 130 135 140	
AAA TGT GAC AAG CCG AGG CGG TGATGAATGA ATGAGGATCC	473
Lys Cys Asp Lys Pro Arg Arg	
145	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 605 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

121

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 13..588

(D) OTHER INFORMATION: /product= "VEGF₁₆₅-encoding DNA"

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 13..90

(D) OTHER INFORMATION: /product= "leader sequence-encoding DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGATCCGAAA CC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC CTT	48
Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu	
1 5 10	
GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC	96
Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro	
15 20 25	
ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC ATG	144
Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met	
30 35 40	
GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG GAC	192
Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp	
45 50 55 60	
ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC	240
Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser	
65 70 75	
TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG	288
Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu	
80 85 90	
GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG CGG	336
Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg	
95 100 105	
ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA CAG	384
Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln	
110 115 120	
CAC AAC AAA TGT GAA TGC AGA CCA AAG AAA GAT AGA GCA AGA CAA GAA	432
His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu	
125 130 135 140	
AAT CCC TGT GGG CCT TGC TCA GAG CGG AGA AAG CAT TTG TTT GTA CAA	480
Asn Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln	
145 150 155	

122

GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC 528
 Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
 160 165 170

AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC AAG 576
 Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys
 175 180 185

CCG AGG CGG TGATGAATGA ATGAGGATCC 605
 Pro Arg Arg
 190

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 677 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 13..657
- (D) OTHER INFORMATION: /product= "VEGF₁₈₉-encoding DNA"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 13..90
- (D) OTHER INFORMATION: /product= "leader sequence-encoding DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

GGATCCGAAA CC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC CTT 48
 Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu
 1 5 10

GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC 96
 Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro
 15 20 25

ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC ATG 144
 Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met
 30 35 40

GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG GAC 192
 Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp
 45 50 55 60

ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC 240
 Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
 65 70 75

TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG 288
 Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu
 80 85 90

123

GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG CGG	336
Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg	
95 100 105	
ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA CAG	384
Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln	
110 115 120	
CAC AAC AAA TGT GAA TGC AGA CCA AAG AAG GAT AGA GCA AGA CAA GAA	432
His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu	
125 130 135 140	
AAA AAA TCA GTT CGA GGA AAG GGA AAG GGG CAA AAA CGA AAG CGC AAG	480
Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys	
145 150 155	
AAA TCC CGG TAT AAG TCC TGG AGC GTT CCC TGT GGG CCT TGC TCA GAG	528
Lys Ser Arg Tyr Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu	
160 165 170	
CGG AGA AAG CAT TTG TTT GTA CAA GAT CCG CAG ACG TGT AAA TGT TCC	576
Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser	
175 180 185	
TGC AAA AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC	624
Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn	
190 195 200	
GAA CGT ACT TGC AGA TGT GAC AAG CCG AGG CGG TGATGAATGA ATGAGGATCC	677
Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg	
205 210 215	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 728 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 13..711
- (D) OTHER INFORMATION: /product= "VEGF₂₀₆-encoding DNA"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 13..90
- (D) OTHER INFORMATION: /product= leader sequence encoding DNA

124

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGATCCGAAA CC ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC CTT Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu 1 5 10	48
GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC Ala Leu Leu Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro 15 20 25	96
ATG GCA GAA GGA GGA GGG CAG AAT CAT CAC GAA GTG GTG AAG TTC ATG Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met 30 35 40	144
GAT GTC TAT CAG CGC AGC TAC TGC CAT CCA ATC GAG ACC CTG GTG GAC Asp Val Tyr Gln Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp 45 50 55 60	192
ATC TTC CAG GAG TAC CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser 65 70 75	240
TGT GTG CCC CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu 80 85 90	288
GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG CGG Glu Cys Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg 95 100 105	336
ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG ATG AGC TTC CTA CAG Ile Lys Pro His Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln 110 115 120	384
CAC AAC AAA TGT GAA TGC AGA CCA AAG AAG GAT AGA GCA AGA CAA GAA His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu 125 130 135 140	432
AAA AAA TCA GTT CGA GGA AAG GGA AAG GGG CAA AAA CGA AAG CGC AAG Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys 145 150 155	480
AAA TCC CGG TAT AAG TCC TGG AGC GTT TAC GTT GGT GCC CGC TGC TGT Lys Ser Arg Tyr Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys 160 165 170	528
CTA ATG CCC TGG AGC CTC CCT GGC CCC CAT CCC TGT GGG CCT TGC TCA Leu Met Pro Trp Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser 175 180 185	576
GAG CGG AGA AAG CAT TTG TTT GTA CAA GAT CCG CAG ACG TGT AAA TGT Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys 190 195 200	624
TCC TGC AAA AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu 205 210 215 220	672

125

AAC GAA CGT ACT TGC AGA TGT GAC AAG CCG AGG CGG TGATGAATGA 718
 Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 225 230 235

ATGAGGATCC 728

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 208 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..627
- (D) OTHER INFORMATION: /note "human HBEGF precursor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val
 1 5 10 15
 Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly
 20 25 30
 Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Pro Thr Val Ser Thr Asp
 35 40 45
 Gln Leu Leu Pro Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu
 50 55 60
 Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro
 65 70 75 80
 Gln Ala Leu Ala Thr Pro Asn Lys Glu Glu His Gly Lys Arg Lys Lys
 85 90 95
 Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr
 100 105 110
 Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg
 115 120 125
 Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His
 130 135 140
 Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr
 145 150 155 160
 Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
 165 170 175

126

Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
 180 185 190

Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His
 195 200 205

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /note "human mature HBEGF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Val Thr Leu Ser Ser Lys Pro Gln Ala Leu Ala Thr Pro Asn Lys
 1 5 10 15
 Glu Glu His Gly Lys Arg Lys Lys Lys Gly Lys Gly Leu Gly Lys Lys
 20 25 30
 Arg Asp Pro Cys Leu Arg Lys Tyr Lys Asp Phe Cys Ile His Gly Glu
 35 40 45
 Cys Lys Tyr Val Lys Glu Leu Arg Ala Pro Ser Cys Ile Cys His Pro
 50 55 60
 Gly Tyr His Gly Glu Arg Cys His Gly Leu Ser Leu Pro
 65 70 75

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 208 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /note "monkey HBEGF precursor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Leu Ala Ala Val
 1 5 10 15
 Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Gln Leu Arg Arg Gly
 20 25 30

127

Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Ser Thr Gly Ser Thr Asp
 35 40 45
 Gln Leu Leu Arg Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu
 50 55 60
 Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro
 65 70 75 80
 Gln Ala Leu Ala Thr Pro Ser Lys Glu Glu His Gly Lys Arg Lys Lys
 85 90 95
 Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr
 100 105 110
 Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg
 115 120 125
 Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His
 130 135 140
 Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr
 145 150 155 160
 Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
 165 170 175
 Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
 180 185 190
 Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His
 195 200 205

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 208 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /note "rat HBEGF precursor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val
 1 5 10 15
 Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly
 20 25 30

128

Leu Ala Ala Ala Thr Ser Asn Pro Asp Pro Pro Thr Gly Thr Thr Asn
 35 40 45
 Gln Leu Leu Pro Thr Gly Ala Asp Arg Ala Gln Glu Val Gln Asp Leu
 50 55 60
 Glu Gly Thr Asp Leu Asp Leu Phe Lys Val Ala Phe Ser Ser Lys Pro
 65 70 75 80
 Gln Ala Leu Ala Thr Pro Gly Lys Glu Lys Asn Gly Lys Lys Lys Arg
 85 90 95
 Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Lys Lys Tyr
 100 105 110
 Lys Asp Tyr Cys Ile His Gly Glu Cys Arg Tyr Leu Lys Glu Leu Arg
 115 120 125
 Ile Pro Ser Cys His Cys Leu Pro Gly Tyr His Gly Gln Arg Cys His
 130 135 140
 Gly Leu Thr Leu Pro Val Glu Asn Pro Leu Tyr Thr Tyr Asp His Thr
 145 150 155 160
 Thr Val Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu
 165 170 175
 Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr
 180 185 190
 Asp Leu Glu Ser Glu Glu Lys Val Lys Leu Gly Met Ala Ser Ser His
 195 200 205

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 627 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..627
- (D) OTHER INFORMATION: /note "human HBEGF precursor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG AAG CTG CTG CCG TCG GTG GTG CTG AAG CTC TTT CTG GCT GCA GTT	48
Met Lys Leu Leu Pro Ser Val Val Leu Lys Leu Phe Leu Ala Ala Val	
1 5 10 15	
CTC TCG GCA CTG GTG ACT GGC GAG AGC CTG GAG CGG CTT CGG AGA GGG	96
Leu Ser Ala Leu Val Thr Gly Glu Ser Leu Glu Arg Leu Arg Arg Gly	
20 25 30	

129

CTA GCT GCT GGA ACC AGC AAC CCG GAC CCT CCC ACT GTA TCC ACG GAC Leu Ala Ala Gly Thr Ser Asn Pro Asp Pro Pro Thr Val Ser Thr Asp 35 40 45	144
CAG CTG CTA CCC CTA GGA GGC GGC CGG GAC CGG AAA GTC CGT GAC TTG Gln Leu Leu Pro Leu Gly Gly Gly Arg Asp Arg Lys Val Arg Asp Leu 50 55 60	192
CAA GAG GCA GAT CTG GAC CTT TTG AGA GTC ACT TTA TCC TCC AAG CCA Gln Glu Ala Asp Leu Asp Leu Leu Arg Val Thr Leu Ser Ser Lys Pro 65 70 75 80	240
CAA GCA CTG GCC ACA CCA AAC AAG GAG GAG CAC GGG AAA AGA AAG AAG Gln Ala Leu Ala Thr Pro Asn Lys Glu Glu His Gly Lys Arg Lys Lys 85 90 95	288
AAA GGC AAG GGG CTA GGG AAG AAG AGG GAC CCA TGT CTT CGG AAA TAC Lys Gly Lys Gly Leu Gly Lys Lys Arg Asp Pro Cys Leu Arg Lys Tyr 100 105 110	336
AAG GAC TTC TGC ATC CAT GGA GAA TGC AAA TAT GTG AAG GAG CTC CGG Lys Asp Phe Cys Ile His Gly Glu Cys Lys Tyr Val Lys Glu Leu Arg 115 120 125	384
GCT CCC TCC TGC ATC TGC CAC CCG GGT TAC CAT GGA GAG AGG TGT CAT Ala Pro Ser Cys Ile Cys His Pro Gly Tyr His Gly Glu Arg Cys His 130 135 140	432
GGG CTG AGC CTC CCA GTG GAA AAT CGC TTA TAT ACC TAT GAC CAC ACA Gly Leu Ser Leu Pro Val Glu Asn Arg Leu Tyr Thr Tyr Asp His Thr 145 150 155 160	480
ACC ATC CTG GCC GTG GTG GCT GTG GTG CTG TCA TCT GTC TGT CTG CTG Thr Ile Leu Ala Val Val Ala Val Val Leu Ser Ser Val Cys Leu Leu 165 170 175	528
GTC ATC GTG GGG CTT CTC ATG TTT AGG TAC CAT AGG AGA GGA GGT TAT Val Ile Val Gly Leu Leu Met Phe Arg Tyr His Arg Arg Gly Gly Tyr 180 185 190	576
GAT GTG GAA AAT GAA GAG AAA GTG AAG TTG GGC ATG ACT AAT TCC CAC Asp Val Glu Asn Glu Glu Lys Val Lys Leu Gly Met Thr Asn Ser His 195 200 205	624
TGA	627

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 155 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

130

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Ala Glu Gly Glu Ile Thr Thr Phe Thr Ala Leu Thr Glu Lys Phe
 1           5           10           15

Asn Leu Pro Pro Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser
          20           25           30

Asn Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly
      35           40           45

Thr Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu
 50           55           60

Ser Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu
65           70           75           80

Ala Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu
          85           90           95

Glu Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr
100           105           110

Ile Ser Lys Lys His Ala Glu Lys Asn Trp Phe Val Gly Leu Lys Lys
115           120           125

Asn Gly Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala
130           135           140

Ile Leu Phe Leu Pro Leu Pro Val Ser Ser Asp
145           150           155

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 155 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
 1           5           10           15

Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
20           25           30

```

131

Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
35 40 45

Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
50 55 60

Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
65 70 75 80

Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys
85 90 95

Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
100 105 110

Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
115 120 125

Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
130 135 140

Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
145 150 155

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 239 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Gly Leu Ile Trp Leu Leu Leu Ser Leu Leu Glu Pro Gly Trp
1 5 10 15

Pro Ala Ala Gly Pro Gly Ala Arg Leu Arg Arg Asp Ala Gly Gly Arg
20 25 30

Gly Gly Val Tyr Glu His Leu Gly Gly Ala Pro Arg Arg Arg Lys Leu
35 40 45

Tyr Cys Ala Thr Lys Tyr His Leu Gln Leu His Pro Ser Gly Arg Val
50 55 60

Asn Gly Ser Leu Glu Asn Ser Ala Tyr Ser Ile Leu Glu Ile Thr Ala
65 70 75 80

Val Glu Val Gly Ile Val Ala Ile Arg Gly Leu Phe Ser Gly Arg Tyr
85 90 95

132

Leu Ala Met Asn Lys Arg Gly Arg Leu Tyr Ala Ser Glu His Tyr Ser
 100 105 110
 Ala Glu Cys Glu Phe Val Glu Arg Ile His Glu Leu Gly Tyr Asn Thr
 115 120 125
 Tyr Ala Ser Arg Leu Tyr Arg Thr Val Ser Ser Thr Pro Gly Ala Arg
 130 135 140
 Arg Gln Pro Ser Ala Glu Arg Leu Trp Tyr Val Ser Val Asn Gly Lys
 145 150 155 160
 Gly Arg Pro Arg Arg Gly Phe Lys Thr Arg Arg Thr Gln Lys Ser Ser
 165 170 175
 Leu Phe Leu Pro Arg Val Leu Asp His Arg Asp His Glu Met Val Arg
 180 185 190
 Gln Leu Gln Ser Gly Leu Pro Arg Pro Pro Gly Lys Gly Val Gln Pro
 195 200 205
 Arg Arg Arg Arg Gln Lys Gln Ser Pro Asp Asn Leu Glu Pro Ser His
 210 215 220
 Val Gln Ala Ser Arg Leu Gly Ser Gln Leu Glu Ala Ser Ala His
 225 230 235

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 206 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Ser Gly Pro Gly Thr Ala Ala Val Ala Leu Leu Pro Ala Val Leu
 1 5 10 15
 Leu Ala Leu Leu Ala Pro Trp Ala Gly Arg Gly Gly Ala Ala Ala Pro
 20 25 30
 Thr Ala Pro Asn Gly Thr Leu Glu Ala Glu Leu Glu Arg Arg Trp Glu
 35 40 45
 Ser Leu Val Ala Leu Ser Leu Ala Arg Leu Pro Val Ala Ala Gln Pro
 50 55 60
 Lys Glu Ala Ala Val Gln Ser Gly Ala Gly Asp Tyr Leu Leu Gly Ile
 65 70 75 80

Lys	Arg	Leu	Arg	Arg	Leu	Tyr	Cys	Asn	Val	Gly	Ile	Gly	Phe	His	Leu	
					85				90						95	
Gln	Ala	Leu	Pro	Asp	Gly	Arg	Ile	Gly	Gly	Ala	His	Ala	Asp	Thr	Arg	
			100					105						110		
Asp	Ser	Leu	Leu	Glu	Leu	Ser	Pro	Val	Glu	Arg	Gly	Val	Val	Ser	Ile	
			115				120					125				
Phe	Gly	Val	Ala	Ser	Arg	Phe	Phe	Val	Ala	Met	Ser	Ser	Lys	Gly	Lys	
			130				135					140				
Leu	Tyr	Gly	Ser	Pro	Phe	Phe	Thr	Asp	Glu	Cys	Thr	Phe	Lys	Glu	Ile	
145						150				155					160	
Leu	Leu	Pro	Asn	Asn	Tyr	Asn	Ala	Tyr	Glu	Ser	Tyr	Lys	Tyr	Pro	Gly	
				165					170					175		
Met	Phe	Ile	Ala	Leu	Ser	Lys	Asn	Gly	Lys	Thr	Lys	Lys	Gly	Asn	Arg	
			180					185					190			
Val	Ser	Pro	Thr	Met	Lys	Val	Thr	His	Phe	Leu	Pro	Arg	Leu			
			195				200					205				

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 268 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1 5 10 15
Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
20 25 30
Gly Pro Ala Ala Thr Asp Arg Asn Pro Ile Gly Ser Ser Ser Arg Gln
35 40 45
Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
50 55 60
Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65 70 75 80
Trp Ser Pro Ser Gly Arg Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly
85 90 95

134

Ile Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser
 100 105 110
 His Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln
 115 120 125
 Gly Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met
 130 135 140
 Ser Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys
 145 150 155 160
 Lys Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser
 165 170 175
 Ala Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu
 180 185 190
 Asn Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro
 195 200 205
 Gln His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln
 210 215 220
 Pro Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Asn Pro Pro
 225 230 235 240
 Ser Pro Ile Lys Ser Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr
 245 250 255
 Asn Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
 260 265

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 198 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ser Arg Gly Ala Gly Arg Leu Gln Gly Thr Leu Trp Ala Leu Val
 1 5 10 15
 Phe Leu Gly Ile Leu Val Gly Met Val Val Pro Ser Pro Ala Gly Thr
 20 25 30
 Arg Ala Asn Asn Thr Leu Leu Asp Ser Arg Gly Trp Gly Thr Leu Leu
 35 40 45

135

Ser Arg Ser Arg Ala Gly Leu Ala Gly Glu Ile Ala Gly Val Asn Trp
 50 55 60
 Glu Ser Gly Tyr Leu Val Gly Ile Lys Arg Gln Arg Arg Leu Tyr Cys
 65 70 75 80
 Asn Val Gly Ile Gly Phe His Leu Gln Val Leu Pro Asp Gly Arg Ile
 85 90 95
 Ser Gly Thr His Glu Glu Asn Pro Tyr Ser Leu Leu Glu Ile Ser Thr
 100 105 110
 Val Glu Arg Gly Val Val Ser Leu Phe Gly Val Arg Ser Ala Leu Phe
 115 120 125
 Val Ala Met Asn Ser Lys Gly Arg Leu Tyr Ala Thr Pro Ser Phe Gln
 130 135 140
 Glu Glu Cys Lys Phe Arg Glu Thr Leu Leu Pro Asn Asn Tyr Asn Ala
 145 150 155 160
 Tyr Glu Ser Asp Leu Tyr Gln Gly Thr Tyr Ile Ala Leu Ser Lys Tyr
 165 170 175
 Gly Arg Val Lys Arg Gly Ser Lys Val Ser Pro Ile Met Thr Val Thr
 180 185 190
 His Phe Leu Pro Arg Ile
 195

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (D) OTHER INFORMATION: /note= "FGF-7"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met His Lys Trp Ile Leu Thr Trp Ile Leu Pro Thr Leu Leu Tyr Arg
 1 5 10 15
 Ser Cys Phe His Ile Ile Cys Leu Val Gly Thr Ile Ser Leu Ala Cys
 20 25 30
 Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys Ser Ser
 35 40 45
 Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile
 50 55 60

136

Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp
 65 70 75 80
 Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn
 85 90 95
 Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly
 100 105 110
 Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr
 115 120 125
 Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu
 130 135 140
 Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly
 145 150 155 160
 Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly
 165 170 175
 Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala
 180 185 190
 Ile Thr

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 215 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
 1 5 10 15
 Val Leu Cys Leu Gln Ala Gln Val Thr Val Gln Ser Ser Pro Asn Phe
 20 25 30
 Thr Gln His Val Arg Glu Gln Ser Leu Val Thr Asp Gln Leu Ser Arg
 35 40 45
 Arg Leu Ile Arg Thr Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys His
 50 55 60
 Val Gln Val Leu Ala Asn Lys Arg Ile Asn Ala Met Ala Glu Asp Gly
 65 70 75 80

137

Asp Pro Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly Ser Arg
 85 90 95
 Val Arg Val Arg Gly Ala Glu Thr Gly Leu Tyr Ile Cys Met Asn Lys
 100 105 110
 Lys Gly Lys Leu Ile Ala Lys Ser Asn Gly Lys Gly Lys Asp Cys Val
 115 120 125
 Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Thr Ala Leu Gln Asn Ala
 130 135 140
 Lys Tyr Glu Gly Trp Tyr Met Ala Phe Thr Arg Lys Gly Arg Pro Arg
 145 150 155 160
 Lys Gly Ser Lys Thr Arg Gln His Gln Arg Glu Val His Phe Met Lys
 165 170 175
 Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg Phe Glu
 180 185 190
 Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser Gln Arg
 195 200 205
 Thr Trp Ala Pro Glu Pro Arg
 210 215

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 208 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "FGF-9"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Pro Leu Gly Glu Val Gly Asn Tyr Phe Gly Val Gln Asp Ala
 1 5 10 15
 Val Pro Phe Gly Asn Val Pro Val Leu Pro Val Asp Ser Pro Val Leu
 20 25 30
 Leu Ser Asp His Leu Gly Gln Ser Glu Ala Gly Gly Leu Pro Arg Gly
 35 40 45
 Pro Ala Val Thr Asp Leu Asp His Leu Lys Gly Ile Leu Arg Arg Arg
 50 55 60
 Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly
 65 70 75 80

138

Thr Ile Gln Gly Thr Arg Lys Asp His Ser Arg Phe Gly Ile Leu Glu
 85 90 95
 Phe Ile Ser Ile Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser
 100 105 110
 Gly Leu Tyr Leu Gly Met Asn Glu Lys Gly Glu Leu Tyr Gly Ser Glu
 115 120 125
 Lys Leu Thr Gln Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp
 130 135 140
 Tyr Asn Thr Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg
 145 150 155 160
 Arg Tyr Tyr Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Glu Gly Thr
 165 170 175
 Arg Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val
 180 185 190
 Asp Pro Asp Lys Val Pro Glu Leu Tyr Lys Asp Ile Leu Ser Gln Ser
 195 200 205

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 804 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..804

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..804
- (D) OTHER INFORMATION: /note= "Nucleotide sequence
corresponding to the clone M13 mp18-G4 in Example I.B.2."

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 46..804
- (D) OTHER INFORMATION: /product= ""Saporin""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCA TGG ATC CTG CTT CAA TTT TCA GCT TGG ACA ACA ACT GAT GCG GTC
 Ala Trp Ile Leu Leu Gln Phe Ser Ala Trp Thr Thr Thr Asp Ala Val
 -15 -10 -5 1

139

ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser 5 10 15	96
TCT TTT GTG GAT AAA ATC CGA AAC AAT GTA AAG GAT CCA AAC CTG AAA Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys 20 25 30	144
TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys 35 40 45	192
TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly 50 55 60 65	240
CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn 70 75 80	288
ACG AAT GTT AAT CGG GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC Thr Asn Val Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala 85 90 95	336
GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala 100 105 110	384
TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile 115 120 125	432
ACA CAG GGA GAT AAA AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu 130 135 140 145	480
CTT TTG ACG TTC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA Leu Leu Thr Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys 150 155 160	528
AAC GAA GCT AGG TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val 165 170 175	576
GCA CGA TTT AGG TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn 180 185 190	624
AAG TTC GAC TCG GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg 195 200 205	672
AAG ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn 210 215 220 225	720

140

AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG 768
 Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu
 230 235 240

CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG 804
 Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys
 245 250

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 804 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..804

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..804
- (D) OTHER INFORMATION: /note= "Nucleotide sequence
corresponding to the clone M13 mp18-G1 in Example I.B.2."

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 46..804
- (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCA TGG ATC CTG CTT CAA TTT TCA GCT TGG ACA ACA ACT GAT GCG GTC 48
 Ala Trp Ile Leu Leu Gln Phe Ser Ala Trp Thr Thr Thr Asp Ala Val
 -15 -10 -5 1

ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA 96
 Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser
 5 10 15

TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA 144
 Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys
 20 25 30

TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA 192
 Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys
 35 40 45

TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC 240
 Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly
 50 55 60 65

141

CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn 70 75 80	288
ACG AAT GTT AAT CGG GCA TAT TAC TTC AGA TCA GAA ATT ACT TCC GCC Thr Asn Val Asn Arg Ala Tyr Tyr Phe Arg Ser Glu Ile Thr Ser Ala 85 90 95	336
GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala 100 105 110	384
TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile 115 120 125	432
ACA CAG GGA GAT AAA TCA AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu 130 135 140 145	480
CTT TTG ACG TCC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA Leu Leu Thr Ser Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys 150 155 160	528
AAC GAA GCT AGG TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val 165 170 175	576
GCA CGA TTT CGG TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn 180 185 190	624
AAG TTC GAC TCG GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg 195 200 205	672
AAG ATT TCT ACG GCA ATA TAC GGA GAT GCC AAA AAC GGC GTG TTT AAT Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn 210 215 220 225	720
AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu 230 235 240	768
CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys 245 250	804

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 804 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..804

(ix) FEATURE:

(A) NAME/KEY: misc feature

(B) LOCATION: 1..804

(D) OTHER INFORMATION: /note= "Nucleotide sequence
corresponding to the clone M13 mp18-G2 in Example I.B.2."

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 46..804

(D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCA TGG ATC CTG CTT CAA TTT TCA GCT TGG ACA ACA ACT GAT GCG GTC 48
Ala Trp Ile Leu Leu Gln Phe Ser Ala Trp Thr Thr Thr Asp Ala Val
-15 -10 -5 1

ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACT GCG GGT CAA TAC TCA 96
Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser
5 10 15

TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA 144
Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys
20 25 30

TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAT AAA 192
Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Asp Lys
35 40 45

TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC 240
Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly
50 55 60 65

CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC 288
Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn
70 75 80

ACG AAT GTT AAT CGG GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC 336
Thr Asn Val Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala
85 90 95

GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT 384
Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala
100 105 110

TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA 432
Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile
115 120 125

ACA CAG GGA GAT AAA AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA 480
Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu
130 135 140 145

143

CTT TTG ACG TTC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA Leu Leu Thr Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys 150 155 160	528
AAC GAA GCT AGG TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val 165 170 175	576
GCA CGA TTT AGG TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn 180 185 190	624
AAG TTC GAC TCG GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg 195 200 205	672
AAG ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn 210 215 220 225	720
AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu 230 235 240	768
CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys 245 250	804

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 804 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..804

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..804
- (D) OTHER INFORMATION: /note= "Nucleotide sequence
corresponding to the clone M13 mp18-G7 in Example I.B.2."

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 46..804
- (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

144

GCA TGG ATC CTG CTT CAA TTT TCA GCT TGG ACA ACA ACT GAT GCG GTC Ala Trp Ile Leu Leu Gln Phe Ser Ala Trp Thr Thr Thr Asp Ala Val -15 -10 -5 1	48
ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser 5 10 15	96
TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys 20 25 30	144
TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys 35 40 45	192
TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly 50 55 60 65	240
CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn 70 75 80	288
ACG AAT GTT AAT CGG GCA TAT TAC TTC AGA TCA GAA ATT ACT TCC GCC Thr Asn Val Asn Arg Ala Tyr Tyr Phe Arg Ser Glu Ile Thr Ser Ala 85 90 95	336
GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala 100 105 110	384
TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile 115 120 125	432
ACA CAG GGA GAT AAA TCA AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu 130 135 140 145	480
CTT TTG ACG TCC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA Leu Leu Thr Ser Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys 150 155 160	528
AAC GAA GCT AGA TTC CTT CTT ATC GCT ATT CAG ATG ACG GCT GAG GCA Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Ala 165 170 175	576
GCA CGA TTT AGG TAC ATA CAA AAC TTG GTA ATC AAG AAC TTT CCC AAC Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Ile Lys Asn Phe Pro Asn 180 185 190	624
AAG TTC AAC TCG GAA AAC AAA GTG ATT CAG TTT GAG GTT AAC TGG AAA Lys Phe Asn Ser Glu Asn Lys Val Ile Gln Phe Glu Val Asn Trp Lys 195 200 205	672

145

AAA ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT 720
 Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn
 210 215 220 225

AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG 768
 Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu
 230 235 240

CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG 804
 Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys
 245 250

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 804 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..804

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..804
- (D) OTHER INFORMATION: /note= "Nucleotide sequence
 corresponding to the clone M13 mp18-G9 in Example I.B.2."

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 46..804
- (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCA TGG ATC CTG CTT CAA TTT TCA GCT TGG ACA ACA ACT GAT GCG GTC 48
 Ala Trp Ile Leu Leu Gln Phe Ser Ala Trp Thr Thr Thr Asp Ala Val
 -15 -10 -5 1

ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA 96
 Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser
 5 10 15

TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA 144
 Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys
 20 25 30

TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA 192
 Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys
 35 40 45

146

TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC	240
Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly	
50 55 60 65	
CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC	288
Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn	
70 75 80	
ACG AAT GTT AAT CGG GCA TAT TAC TTC AGA TCA GAA ATT ACT TCC GCC	336
Thr Asn Val Asn Arg Ala Tyr Tyr Phe Arg Ser Glu Ile Thr Ser Ala	
85 90 95	
GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT	384
Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala	
100 105 110	
TTA GAA TAC ACA GAA GAT TAT CAG TCG ATT GAA AAG AAT GCC CAG ATA	432
Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile	
115 120 125	
ACA CAA GGA GAT CAA AGT AGA AAA GAA CTC GGG TTG GGG ATT GAC TTA	480
Thr Gln Gly Asp Gln Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu	
130 135 140 145	
CTT TCA ACG TCC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA	528
Leu Ser Thr Ser Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys	
150 155 160	
GAC GAA GCT AGA TTC CTT CTT ATC GCT ATT CAG ATG ACG GCT GAG GCA	576
Asp Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Ala	
165 170 175	
GCG CGA TTT AGG TAC ATA CAA AAC TTG GTA ATC AAG AAC TTT CCC AAC	624
Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Ile Lys Asn Phe Pro Asn	
180 185 190	
AAG TTC AAC TCG GAA AAC AAA GTG ATT CAG TTT GAG GTT AAC TGG AAA	672
Lys Phe Asn Ser Glu Asn Lys Val Ile Gln Phe Glu Val Asn Trp Lys	
195 200 205	
AAA ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT	720
Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn	
210 215 220 225	
AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG	768
Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu	
230 235 240	
CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG	804
Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys	
245 250	

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid

147

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..7
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Pro Lys Lys Arg Lys Val Glu
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..8
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Pro Pro Lys Lys Ala Arg Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..9
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Ala Ala Lys Arg Val Lys Leu Asp
1 5

(2) INFORMATION FOR SEQ ID NO:27:

148

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..5
 (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Lys Arg Pro Arg Pro
 1 5

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..5
 (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Lys Ile Pro Ile Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..9
 (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Lys Arg Lys Arg Lys Ser
 1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..9
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Lys Arg Val Ala Lys Arg Lys Leu
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..9
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ser His Trp Lys Gln Lys Arg Lys Phe
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..8
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

150

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Pro Leu Leu Lys Lys Ile Lys Gln
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..7
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Pro Gln Pro Lys Lys Lys Pro
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..15
- (D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Pro Gly Lys Arg Lys Lys Glu Met Thr Lys Gln Lys Glu Val Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS

151

(B) LOCATION: 1..12

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
 1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..7

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Asn Tyr Lys Lys Pro Lys Leu
 1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..7

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

His Phe Lys Asp Pro Lys Arg
 1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..7

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ala Pro Arg Arg Arg Lys Leu

1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..6

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ile Lys Arg Leu Arg Arg

1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..6

(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ile Lys Arg Gln Arg Arg

1 5

(2) INFORMATION FOR SEQ ID NO:41:

153

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /product= nuclear translocation sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ile Arg Val Arg Arg
1 5

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: /note= "Cytoplasmic Translocation
Signal"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Lys Asp Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: /note= "Cytoplasmic Translocation
Signal"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Arg Asp Glu Leu
1

154

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Cytoplasmic Translocation Signal"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Lys Glu Glu Leu
1

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Endosome-disruptive peptide INF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Leu Phe Glu Ala Ile Glu Gly Phe Ile Glu Asn Gly Trp Glu Gly
1 5 10 15
Met Ile Asp Gly Gly Gly Cys
20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Endosome-disruptive peptide INF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Gly Leu Phe Glu Ala Ile Glu Gly Phe Ile Glu Asn Gly Trp Glu Gly
1 5 10 15
Met Ile Asp Gly Trp Tyr Gly Cys
20

155

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..26
- (A) NAME/KEY: Gly₄Ser with NcoI ends

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CCATGGGCGG CGGCGGCTCT GCCATGG

27

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..41
- (A) NAME/KEY: (Gly₄Ser)₂ with NcoI ends

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CCATGGGCGG CGGCGGCTCT GGCGGGCGCG GCTCTGCCAT GG

42

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..74
- (A) NAME/KEY: (Ser₄Gly)₄ with NcoI ends

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CCATGGCCTC GTCGTCGTCG GGCTCGTCGT CGTCGGGCTC GTCGTCGTCG GGCTCGTCGT

60

156

CGTCGGGCGC CATGG

75

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..45
- (A) NAME/KEY: (Ser,Gly)₂

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCATGGCCTC GTCGTCGTCG GGCTCGTCGT CGTCGGGCGC CATGG

45

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..8
- (D) OTHER INFORMATION: /product= Flexible linker

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ala Ala Pro Ala Ala Ala Pro Ala
1 5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 465 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..465

(ix) FEATURE:

157

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..465
 (D) OTHER INFORMATION: /product= "bGFG"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC	465
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser	
145 150 155	

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1230 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

158

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..1230

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..465
 (D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 472..1230
 (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATG GCT GCT GGT TCT ATC ACT ACT CTG CCG GCT CTG CCG GAA GAC GGT	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGT TCT GGT GCT TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GTC ACA TCA	480
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Val Thr Ser	
145 150 155 160	

159

ATC ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe 165 170 175	528
GTG GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly 180 185 190	576
GGT ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu 195 200 205	624
AGA ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys 210 215 220	672
CGC GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn 225 230 235 240	720
GTT AAT CGG GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA Val Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu 245 250 255	768
ACC GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu 260 265 270	816
TAC ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln 275 280 285	864
GGA GAT AAA AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu 290 295 300	912
ACG TTC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA Thr Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu 305 310 315 320	960
GCT AGG TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg 325 330 335	1008
TTT AGG TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe 340 345 350	1056
GAC TCG GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile 355 360 365	1104
TCT ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp 370 375 380	1152

160

TAT GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG 1200
 Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met
 385 390 395 400

GGA CTC CTT ATG TAT TTG GGC AAA CCA AAG 1230
 Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys
 405 410

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION: 6..11
- (D) OTHER INFORMATION: /standard_name= "EcoRI Restriction Site"

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 12..30
- (D) OTHER INFORMATION: /function= "N-terminal extension" /product= "Native saporin signal peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTGCAGAATT CGCATGGATC CTGCTTCAAT 30

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION: 6..11
- (D) OTHER INFORMATION: /standard_name= "EcoRI Restriction Site"

(ix) FEATURE:

- (A) NAME/KEY: terminator
- (B) LOCATION: 23..25
- (D) OTHER INFORMATION: /note= "Anti-sense stop codon"

161

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 26..30
- (D) OTHER INFORMATION: /note= "Anti-sense to carboxyl terminus of mature peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55

CTGCAGAATT CGCCTCGTTT GACTACTTTG

30

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

AGGAGTGTCT GCTAACC

17

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TTCTAAATCG GTTACCGATG ACTG

24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CATATGTGTG AGCTACTGTC GCCACCGCTC

30

162

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGATCCGAGC ACCTGGTATA TCGGTGGGGG

30

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGATCCGCCT CGTTTGACTA CTT

23

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(D) OTHER INFORMATION/product= bacteriophage lambda CII ribosome binding site

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GTCGACCAAG CTTGGGCATA CATTCAATCA ATTGTTATCT AAGGAAATAC TTACATATG

59

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(D) OTHER INFORMATION: /product= trp promoter

163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

AATCCCCTG TTGACAATTA ATCATCGAAC TAGTTAACTA GTACGCAGCT TGGCTGCAG 59

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION: 11..16
- (D) OTHER INFORMATION: /standard_name= "Nco I restriction enzyme recognition site."

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /product= "Carboxy terminus of mature FGF protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTAAGAGCG CCATGGAGA 19

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /product= "Carboxy terminus of wild type FGF"

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION: 13..18
- (D) OTHER INFORMATION: /standard_name= "Nco I restriction enzyme recognition site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GCT AAG AGC TGACCATGGA GA 21
Ala Lys Ser

164

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..96
- (D) OTHER INFORMATION: /product= "pFGFNcoI"
/note= "Equals the plasmid pFC80 with native FGF
stop codon removed."

(ix) FEATURE:

- (A) NAME/KEY: misc_recomb
- (B) LOCATION: 29..34
- (D) OTHER INFORMATION: /standard_name= "Nco I restriction enzyme recognition site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GAG ATC CGG CTG AAT 48
 Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Glu Ile Arg Leu Asn
 1 5 10 15

GGT GCA GTT CTG TAC CGG TTT TCC TGT GCC GTC TTT CAG GAC TCC TGAAATCTT 102
 Gly Ala Val Leu Tyr Arg Phe Ser Cys Ala Val Phe Gln Asp Ser
 20 25 30

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..35
- (A) NAME/KEY: Cathepsin B linker

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CCATGGCCCT GGCCCTGGCC CTGGCCCTGG CCATGG

36

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..50
- (A) NAME/KEY: Cathepsin D linker

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CCATGGGCCG ATCGGGCTTC CTGGGCTTCG GCTTCCTGGG CTTCGCCATGG 51

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..95
- (A) NAME/KEY: "Trypsin linker"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CCATGGGCCG ATCGGGCGGT GGGTGCCTG GTAATAGAGT CAGAAGATCA GTCGGAAGCA 60

GCCTGTCTTG CGGTGGTCTC GACCTGCAGG CCATGG 96

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..18
- (D) OTHER INFORMATION: /product= Thrombin substrate linker

166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTG GTG CCG CGC GGC AGC
Leu Val Pro Arg Gly Ser
1 5

18

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..15
- (D) OTHER INFORMATION: /product= Enterokinase substrate linker

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GAC GAC GAC GAC CCA
Asp Asp Asp Asp Lys
1 5

15

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /product= Factor Xa substrate

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

ATC GAA GGT CGT
Ile Glu Gly Arg
1

12

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1260 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

167

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..1260

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..465
(D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 466...501
(D) OTHER INFORMATION: /product= "Cathepsin B linker"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 502..1260
(D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	

168

GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GCC CTG GCC Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Ala Leu Ala 145 150 155 160	480
CTG GCC CTG GCC CTG GCC ATG GTC ACA TCA ATC ACA TTA GAT CTA GTA Leu Ala Leu Ala Leu Ala Met Val Thr Ser Ile Thr Leu Asp Leu Val 165 170 175	528
AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT GTG GAT AAA ATC CGA AAC Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe Val Asp Lys Ile Arg Asn 180 185 190	576
AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT GGT ACC GAC ATA GCC GTG Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly Gly Thr Asp Ile Ala Val 195 200 205	624
ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT AGA ATT AAT TTC CAA AGT Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu Arg Ile Asn Phe Gln Ser 210 215 220	672
TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA CGC GAT AAC TTG TAT GTG Ser Arg Gly Thr Val Ser Leu Gly Leu Lys Arg Asp Asn Leu Tyr Val 225 230 235 240	720
GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT GTT AAT CGG GCA TAT TAC Val Ala Tyr Leu Ala Met Asp Asn Thr Asn Val Asn Arg Ala Tyr Tyr 245 250 255	768
TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA ACC GCC CTT TTC CCA GAG Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu Thr Ala Leu Phe Pro Glu 260 265 270	816
GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA TAC ACA GAA GAT TAT CAG Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu Tyr Thr Glu Asp Tyr Gln 275 280 285	864
TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG GGA GAT AAA AGT AGA AAA Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln Gly Asp Lys Ser Arg Lys 290 295 300	912
GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG ACG TTC ATG GAA GCA GTG Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu Thr Phe Met Glu Ala Val 305 310 315 320	960
AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA GCT AGG TTT CTG CTT ATC Asn Lys Lys Ala Arg Val Val Lys Asn Glu Ala Arg Phe Leu Leu Ile 325 330 335	1008
GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA TTT AGG TAC ATT CAA AAC Ala Ile Gln Met Thr Ala Glu Val Ala Arg Phe Arg Tyr Ile Gln Asn 340 345 350	1056
TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC GAC TCG GAT AAC AAG GTG Leu Val Thr Lys Asn Phe Pro Asn Lys Phe Asp Ser Asp Asn Lys Val 355 360 365	1104

169

ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT TCT ACG GCA ATA TAC GGG Ile Gln Phe Glu Val Ser Trp Arg Lys Ile Ser Thr Ala Ile Tyr Gly 370 375 380	1152
GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT TAT GAT TTC GGG TTT GGA Asp Ala Lys Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe Gly Phe Gly 385 390 395 400	1200
AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG GGA CTC CTT ATG TAT TTG Lys Val Arg Gln Val Lys Asp Leu Gln Met Gly Leu Leu Met Tyr Leu 405 410 415	1248
GGC AAA CCA AAG Gly Lys Pro Lys 420	1260

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1275 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1275

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1..465
- (D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 466..516
- (D) OTHER INFORMATION: /product= "Cathepsin D linker"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 517..1275
- (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly 1 5 10 15	48
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu 20 25 30	96

170

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg 35 40 45	144
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu 50 55 60	192
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn 65 70 75 80	240
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys 85 90 95	288
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr 100 105 110	336
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys 115 120 125	384
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys 130 135 140	432
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GGC CGA TCG Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Gly Arg Ser 145 150 155 160	480
GGC TTC CTG GGC TTC GGC TTC CTG GGC TTC GCC ATG GTC ACA TCA ATC Gly Phe Leu Gly Phe Gly Phe Leu Gly Phe Ala Met Val Thr Ser Ile 165 170 175	528
ACA TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT GTG Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe Val 180 185 190	576
GAT AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT GGT Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly Gly 195 200 205	624
ACC GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT AGA Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu Arg 210 215 220	672
ATT AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA CGC Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys Arg 225 230 235 240	720
GAT AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT GTT Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn Val 245 250 255	768

171

AAT CGG GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA ACC Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu Thr 260 265 270	816
GCC CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA TAC Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu Tyr 275 280 285	864
ACA GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG GGA Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln Gly 290 295 300	912
GAT AAA AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG ACG Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu Thr 305 310 315 320	960
TTC ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA GCT Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu Ala 325 330 335	1008
AGG TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA TTT Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg Phe 340 345 350	1056
AGG TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC GAC Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe Asp 355 360 365	1104
TCG GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT TCT Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile Ser 370 375 380	1152
ACG GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT TAT Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp Tyr 385 390 395 400	1200
GAT TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG GGA Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met Gly 405 410 415	1248
CTC CTT ATG TAT TTG GGC AAA CCA AAG Leu Leu Met Tyr Leu Gly Lys Pro Lys 420 425	1275

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1251 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1251

172

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..465
 (D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 466..492
 (D) OTHER INFORMATION: /product= " Gly,Ser linker"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 493..1251
 (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GGC GGC GGC	480
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Gly Gly Gly	
145 150 155 160	

173

GGC TCT GCC ATG GTC ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC Gly Ser Ala Met Val Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr 165 170 175	528
GCG GGT CAA TAC TCA TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG Ala Gly Gln Tyr Ser Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys 180 185 190	576
GAT CCA AAC CTG AAA TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA Asp Pro Asn Leu Lys Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro 195 200 205	624
CCT TCT AAA GAA AAA TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA Pro Ser Lys Glu Lys Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly 210 215 220	672
ACG GTC TCA CTT GGC CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT Thr Val Ser Leu Gly Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr 225 230 235 240	720
CTT GCA ATG GAT AAC ACG AAT GTT AAT CGG GCA TAT TAC TTC AAA TCA Leu Ala Met Asp Asn Thr Asn Val Asn Arg Ala Tyr Tyr Phe Lys Ser 245 250 255	768
GAA ATT ACT TCC GCC GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT Glu Ile Thr Ser Ala Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr 260 265 270	816
GCA AAT CAG AAA GCT TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA Ala Asn Gln Lys Ala Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu 275 280 285	864
AAG AAT GCC CAG ATA ACA CAG GGA GAT AAA AGT AGA AAA GAA CTC GGG Lys Asn Ala Gln Ile Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly 290 295 300	912
TTG GGG ATC GAC TTA CTT TTG ACG TTC ATG GAA GCA GTG AAC AAG AAG Leu Gly Ile Asp Leu Leu Thr Phe Met Glu Ala Val Asn Lys Lys 305 310 315 320	960
GCA CGT GTG GTT AAA AAC GAA GCT AGG TTT CTG CTT ATC GCT ATT CAA Ala Arg Val Val Lys Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln 325 330 335	1008
ATG ACA GCT GAG GTA GCA CGA TTT AGG TAC ATT CAA AAC TTG GTA ACT Met Thr Ala Glu Val Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr 340 345 350	1056
AAG AAC TTC CCC AAC AAG TTC GAC TCG GAT AAC AAG GTG ATT CAA TTT Lys Asn Phe Pro Asn Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe 355 360 365	1104
GAA GTC AGC TGG CGT AAG ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA Glu Val Ser Trp Arg Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys 370 375 380	1152

174

AAC GGC GTG TTT AAT AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG 1200
Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg
385 390 395 400

CAG GTG AAG GAC TTG CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA 1248
Gln Val Lys Asp Leu Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro
405 410 415

AAG	1251
Lys	

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1266 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1266

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 1..465

(D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 466..507

(D) OTHER INFORMATION: /product= " (Gly₄Ser)₂ linker"

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 508..1266

(D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC 48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
1 5 10 15

GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG 96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
20 25 30

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA 144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
35 40 45

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT 192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
50 55 60

175

CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn 65 70 75 80	240
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys 85 90 95	288
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr 100 105 110	336
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys 115 120 125	384
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys 130 135 140	432
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GGC GGC GGC Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Gly Gly Gly 145 150 155 160	480
GGC TCT GGC GGC GGC GGC TCT GCC ATG GTC ACA TCA ATC ACA TTA GAT Gly Ser Gly Gly Gly Gly Ser Ala Met Val Thr Ser Ile Thr Leu Asp 165 170 175	528
CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT GTG GAT AAA ATC Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe Val Asp Lys Ile 180 185 190	576
CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT GGT ACC GAC ATA Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly Gly Thr Asp Ile 195 200 205	624
GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT AGA ATT AAT TTC Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu Arg Ile Asn Phe 210 215 220	672
CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA CGC GAT AAC TTG Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys Arg Asp Asn Leu 225 230 235 240	720
TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT GTT AAT CGG GCA Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn Val Asn Arg Ala 245 250 255	768
TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA ACC GCC CTT TTC Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu Thr Ala Leu Phe 260 265 270	816
CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA TAC ACA GAA GAT Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu Tyr Thr Glu Asp 275 280 285	864

176

TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG GGA GAT AAA AGT Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln Gly Asp Lys Ser 290 295 300	912
AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG ACG TTC ATG GAA Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu Thr Phe Met Glu 305 310 315 320	960
GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA GCT AGG TTT CTG Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu Ala Arg Phe Leu 325 330 335	1008
CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA TTT AGG TAC ATT Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg Phe Arg Tyr Ile 340 345 350	1056
CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC GAC TCG GAT AAC Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe Asp Ser Asp Asn 355 360 365	1104
AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT TCT ACG GCA ATA Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile Ser Thr Ala Ile 370 375 380	1152
TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT TAT GAT TTC GGG Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe Gly 385 390 395 400	1200
TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG GGA CTC CTT ATG Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met Gly Leu Leu Met 405 410 415	1248
TAT TTG GGC AAA CCA AAG Tyr Leu Gly Lys Pro Lys 420	1266

(2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1320 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1320

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1..465
- (D) OTHER INFORMATION: /product= "bFGF"

177

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 466..561

(D) OTHER INFORMATION: /product= "Trypsin linker"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 562..1320

(D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GGC CGA TCG	480
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Gly Arg Ser	
145 150 155 160	
GGC GGT GGG TGC GCT GGT AAT AGA GTC AGA AGA TCA GTC GGA AGC AGC	528
Gly Gly Gly Cys Ala Gly Asn Arg Val Arg Arg Ser Val Gly Ser Ser	
165 170 175	

178

CTG TCT TGC GGT GGT CTC GAC CTG CAG GCC ATG GTC ACA TCA ATC ACA Leu Ser Cys Gly Gly Leu Asp Leu Gln Ala Met Val Thr Ser Ile Thr 180 185 190	576
TTA GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT GTG GAT Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe Val Asp 195 200 205	624
AAA ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT GGT ACC Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly Gly Thr 210 215 220	672
GAC ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT AGA ATT Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu Arg Ile 225 230 235 240	720
AAT TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA CGC GAT Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys Arg Asp 245 250 255	768
AAC TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT GTT AAT Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn Val Asn 260 265 270	816
CGG GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA ACC GCC Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu Thr Ala 275 280 285	864
CTT TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA TAC ACA Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu Tyr Thr 290 295 300	912
GAA GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG GGA GAT Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln Gly Asp 305 310 315 320	960
AAA AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG ACG TTC Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu Thr Phe 325 330 335	1008
ATG GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA GCT AGG Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu Ala Arg 340 345 350	1056
TTT CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA TTT AGG Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg Phe Arg 355 360 365	1104
TAC ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC GAC TCG Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe Asp Ser 370 375 380	1152
GAT AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT TCT ACG Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile Ser Thr 385 390 395 400	1200

179

GCA ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT TAT GAT	1248
Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp Tyr Asp	
405 410 415	
TTC GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG GGA CTC	1296
Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met Gly Leu	
420 425 430	
CTT ATG TAT TTG GGC AAA CCA AAG	1320
Leu Met Tyr Leu Gly Lys Pro Lys	
435 440	

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1299 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1299

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1..465
- (D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 466..540
- (D) OTHER INFORMATION: /product= "(Ser₄Gly)₄linker"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 541..1299
- (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	

180

GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu 50 55 60	192
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn 65 70 75 80	240
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys 85 90 95	288
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr 100 105 110	336
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys 115 120 125	384
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys 130 135 140	432
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GCC TCG TCG Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Ala Ser Ser 145 150 155 160	480
TCG TCG GGC TCG TCG TCG TCG GGC TCG TCG TCG GGC TCG TCG TCG Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser 165 170 175	528
TCG GGC GCC ATG GTC ACA TCA ATC ACA TTA GAT CTA GTA AAT CCG ACC Ser Gly Ala Met Val Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr 180 185 190	576
GCG GGT CAA TAC TCA TCT TTT GTG GAT AAA ATC CGA AAC AAC GTA AAG Ala Gly Gln Tyr Ser Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys 195 200 205	624
GAT CCA AAC CTG AAA TAC GGT GGT ACC GAC ATA GCC GTG ATA GGC CCA Asp Pro Asn Leu Lys Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro 210 215 220	672
CCT TCT AAA GAA AAA TTC CTT AGA ATT AAT TTC CAA AGT TCC CGA GGA Pro Ser Lys Glu Lys Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly 225 230 235 240	720
ACG GTC TCA CTT GGC CTA AAA CGC GAT AAC TTG TAT GTG GTC GCG TAT Thr Val Ser Leu Gly Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr 245 250 255	768
CTT GCA ATG GAT AAC ACG AAT GTT AAT CGG GCA TAT TAC TTC AAA TCA Leu Ala Met Asp Asn Thr Asn Val Asn Arg Ala Tyr Tyr Phe Lys Ser 260 265 270	816

181

GAA ATT ACT TCC GCC GAG TTA ACC GCC CTT TTC CCA GAG GCC ACA ACT Glu Ile Thr Ser Ala Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr 275 280 285	864
GCA AAT CAG AAA GCT TTA GAA TAC ACA GAA GAT TAT CAG TCG ATC GAA Ala Asn Gln Lys Ala Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu 290 295 300	912
AAG AAT GCC CAG ATA ACA CAG GGA GAT AAA AGT AGA AAA GAA CTC GGG Lys Asn Ala Gln Ile Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly 305 310 315 320	960
TTG GGG ATC GAC TTA CTT TTG ACG TTC ATG GAA GCA GTG AAC AAG AAG Leu Gly Ile Asp Leu Leu Leu Thr Phe Met Glu Ala Val Asn Lys Lys 325 330 335	1008
GCA CGT GTG GTT AAA AAC GAA GCT AGG TTT CTG CTT ATC GCT ATT CAA Ala Arg Val Val Lys Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln 340 345 350	1056
ATG ACA GCT GAG GTA GCA CGA TTT AGG TAC ATT CAA AAC TTG GTA ACT Met Thr Ala Glu Val Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr 355 360 365	1104
AAG AAC TTC CCC AAC AAG TTC GAC TCG GAT AAC AAG GTG ATT CAA TTT Lys Asn Phe Pro Asn Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe 370 375 380	1152
GAA GTC AGC TGG CGT AAG ATT TCT ACG GCA ATA TAC GGG GAT GCC AAA Glu Val Ser Trp Arg Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys 385 390 395 400	1200
AAC GGC GTG TTT AAT AAA GAT TAT GAT TTC GGG TTT GGA AAA GTG AGG Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg 405 410 415	1248
CAG GTG AAG GAC TTG CAA ATG GGA CTC CTT ATG TAT TTG GGC AAA CCA Gln Val Lys Asp Leu Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro 420 425 430	1296
AAG Lys	1299

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1269 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1269

182

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..465
 (D) OTHER INFORMATION: /product= "bFGF"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 466..510
 (D) OTHER INFORMATION: /product= "(Ser₄Gly)₂ linker"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 511..1269
 (D) OTHER INFORMATION: /product= "Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
1 5 10 15	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
GTT GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
CAA GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
CGT TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
GTT ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
AAT ACT TAC CGG TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
CGA ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
130 135 140	
GCT ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC GCC ATG GCC TCG TCG	480
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Ala Ser Ser	
145 150 155 160	

183

TCG TCG GGC TCG TCG TCG TCG GGC GCC ATG GTC ACA TCA ATC ACA TTA Ser Ser Gly Ser Ser Ser Ser Gly Ala Met Val Thr Ser Ile Thr Leu 165 170 175	528
GAT CTA GTA AAT CCG ACC GCG GGT CAA TAC TCA TCT TTT GTG GAT AAA Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe Val Asp Lys 180 185 190	576
ATC CGA AAC AAC GTA AAG GAT CCA AAC CTG AAA TAC GGT GGT ACC GAC Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly Gly Thr Asp 195 200 205	624
ATA GCC GTG ATA GGC CCA CCT TCT AAA GAA AAA TTC CTT AGA ATT AAT Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu Arg Ile Asn 210 215 220	672
TTC CAA AGT TCC CGA GGA ACG GTC TCA CTT GGC CTA AAA CGC GAT AAC Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys Arg Asp Asn 225 230 235 240	720
TTG TAT GTG GTC GCG TAT CTT GCA ATG GAT AAC ACG AAT GTT AAT CGG Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn Val Asn Arg 245 250 255	768
GCA TAT TAC TTC AAA TCA GAA ATT ACT TCC GCC GAG TTA ACC GCC CTT Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu Thr Ala Leu 260 265 270	816
TTC CCA GAG GCC ACA ACT GCA AAT CAG AAA GCT TTA GAA TAC ACA GAA Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu Tyr Thr Glu 275 280 285	864
GAT TAT CAG TCG ATC GAA AAG AAT GCC CAG ATA ACA CAG GGA GAT AAA Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln Gly Asp Lys 290 295 300	912
AGT AGA AAA GAA CTC GGG TTG GGG ATC GAC TTA CTT TTG ACG TTC ATG Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu Thr Phe Met 305 310 315 320	960
GAA GCA GTG AAC AAG AAG GCA CGT GTG GTT AAA AAC GAA GCT AGG TTT Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu Ala Arg Phe 325 330 335	1008
CTG CTT ATC GCT ATT CAA ATG ACA GCT GAG GTA GCA CGA TTT AGG TAC Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg Phe Arg Tyr 340 345 350	1056
ATT CAA AAC TTG GTA ACT AAG AAC TTC CCC AAC AAG TTC GAC TCG GAT Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe Asp Ser Asp 355 360 365	1104
AAC AAG GTG ATT CAA TTT GAA GTC AGC TGG CGT AAG ATT TCT ACG GCA Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile Ser Thr Ala 370 375 380	1152

184

ATA TAC GGG GAT GCC AAA AAC GGC GTG TTT AAT AAA GAT TAT GAT TTC	1200
Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp Tyr Asp Phe	
385 390 395 400	
GGG TTT GGA AAA GTG AGG CAG GTG AAG GAC TTG CAA ATG GGA CTC CTT	1248
Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met Gly Leu Leu	
405 410 415	
ATG TAT TTG GGC AAA CCA AAG	1269
Met Tyr Leu Gly Lys Pro Lys	
420	

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 765 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..762
- (D) OTHER INFORMATION: /product= "Mammalian codon optimized saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

ATG GTG ACC TCC ATC ACC CTG GAC CTG GTG AAC CCC ACC GCC GGC CAG	48
Met Val Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln	
1 5 10 15	
TAC TCC TCC TTC GTG GAC AAG ATC CGC AAC AAC GTG AAG GAC CCC AAC	96
Tyr Ser Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn	
20 25 30	
CTG AAG TAC GGC GGC ACC GAC ATC GCC GTG ATC GGC CCC CCC TCC AAG	144
Leu Lys Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys	
35 40 45	
GAG AAG TTC CTG CGC ATC AAC TTC CAG TCC TCC CGC GGC ACC GTG TCC	192
Glu Lys Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser	
50 55 60	
CTG GGC CTG AAG CGC GAC AAC CTG TAC GTG GTG GCC TAC CTG GCC ATG	240
Leu Gly Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met	
65 70 75 80	
GAC AAC ACC AAC GTG AAC CGC GCC TAC TAC TTC AAG TCC GAG ATC ACC	288
Asp Asn Thr Asn Val Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr	
85 90 95	

185

TCC GCC GAG CTG ACC GCC CTG TTC CCT GAG GCC ACC ACC GCC AAC CAG Ser Ala Glu Leu Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln 100 105 110	336
AAG GCC CTG GAG TAC ACC GAG GAC TAC CAG TCC ATC GAG AAG AAC GCC Lys Ala Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala 115 120 125	384
CAG ATC ACC CAG GGC GAC AAG TCC CGC AAG GAG CTC GGG CTG GGC ATC Gln Ile Thr Gln Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile 130 135 140	432
GAC CTG CTG CTG ACC TTC ATG GAG GCC GTG AAC AAG AAG GCC CGC GTG Asp Leu Leu Leu Thr Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val 145 150 155 160	480
GTG AAG AAC GAG GCC CGC TTC CTG CTG ATC GCC ATC CAG ATG ACC GCC Val Lys Asn Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala 165 170 175	528
GAG GTG GCC CGC TTC CGC TAC ATC CAG AAC CTG GTG ACC AAG AAC TTC Glu Val Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe 180 185 190	576
CCC AAC AAG TTC GAC TCC GAC AAC AAG GTG ATC CAG TTC GAG GTC AGC Pro Asn Lys Phe Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser 195 200 205	624
TGG CGC AAG ATC TCC ACC GCC ATC TAC GGC GAC GCC AAG AAC GGC GTG Trp Arg Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val 210 215 220	672
TTC AAC AAG GAC TAC GAC TTC GGC TTC GGC AAG GTG CGC CAG GTG AAG Phe Asn Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys 225 230 235 240	720
GAC CTG CAG ATG GGC CTG CTG ATG TAC CTG GGC AAG CCC AAG Asp Leu Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys 245 250	762
TAG	765

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1233 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1230

(D) OTHER INFORMATION: /product= "E. coli codon optimized"
FGF-SAP"

FGF-SAP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

ATG GCA GCG GGT TCT ATT ACT ACC CTG CCG GCG CTG GAG GAC GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Gln Asp Gly	
255 260 265 270	
GGT TCT GGC GCT TTC CCA CCG GGC CAC TTT AAG GAC CCG AAA CGC CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
275 280 285	
TAT TGC AAA AAC GGT GGT TTT TTC CTG CGT ATC CAC CCG GAT GGC CGC	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
290 295 300	
GTC GAT GGC GTC CGC GAA AAG TCT GAT CCG CAC ATC AAA CTG CAA TTG	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
305 310 315	
CAA GCA GAG GAA CGC GGT GTT GTA AGC ATC AAG GGC GTT TGC GCG AAT	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
320 325 330	
CGT TAC CTG GCG ATG AAA GAG GAT GGC CGC CTG CTG GCC TCC AAG TGT	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
335 340 345 350	
GTA ACC GAT GAA TGC TTC TTC TTT GAA CGT CTG GAG TCG AAC AAT TAT	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
355 360 365	
AAC ACC TAT CGT AGC CGT AAG TAC ACC TCG TGG TAC GTA GCA TTG AAA	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
370 375 380	
CGC ACC GGT CAG TAC AAA CTG GGT TCG AAG ACG GGT CCA GGT CAG AAA	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	
385 390 395	
GCA ATT CTG TTC CTG CCA ATG TCG GCC AAA TCG GCC ATG GTC ACT TCT	480
Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser Ala Met Val Thr Ser	
400 405 410	
ATC ACG CTG GAT CTG GTC AAC CCG ACC GCT GGT CAG TAC AGC TCG TTT	528
Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr Ser Ser Phe	
415 420 425 430	
GTC GAT AAG ATT CGT AAT AAT GTG AAA GAT CCG AAT TTA AAA TAC GGT	576
Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu Lys Tyr Gly	
435 440 445	

187

GGC ACG GAT ATT GCA GTG ATT GGC CCG CCG TCT AAG GAA AAG TTC TTG Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu Lys Phe Leu 450 455 460	624
CGT ATT AAC TTT CAA AGC TCT CGC GGC ACT GTG TCT CTG GGC TTA AAA Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu Gly Leu Lys 465 470 475	672
CGC GAT AAT TTG TAC GTT GTA GCG TAC CTG GCG ATG GAT AAT ACC AAT Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp Asn Thr Asn 480 485 490	720
GTA AAC CGT GCT TAC TAT TTC AAA AGC GAA ATT ACC TCT GCT GAA CTG Val Asn Arg Ala Tyr Tyr Phe Lys Ser Glu Ile Thr Ser Ala Glu Leu 495 500 505 510	768
ACT GCA TTA TTC CCG GAA GCG ACT ACT GCC AAT CAG AAA GCC CTG GAA Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys Ala Leu Glu 515 520 525	816
TAT ACC GAA GAT TAT CAG TCG ATT GAA AAA AAC GCG CAA ATT ACC CAG Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln Ile Thr Gln 530 535 540	864
GGC GAC AAA TCG CGC AAA GAG TTG GGT CTG GGT ATT GAC CTG CTG CTG Gly Asp Lys Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp Leu Leu Leu 545 550 555	912
ACG TTT ATG GAG GCG GTC AAC AAA AAA GCT CGT GTA GTG AAA AAC GAA Thr Phe Met Glu Ala Val Asn Lys Lys Ala Arg Val Val Lys Asn Glu 560 565 570	960
GCT CGC TTT CTG CTG ATC GCT ATT CAA ATG ACT GCT GAA GTT GCT CGT Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu Val Ala Arg 575 580 585 590	1008
TTC CGT TAC ATT CAG AAC TTG GTT ACT AAG AAC TTT CCG AAC AAA TTC Phe Arg Tyr Ile Gln Asn Leu Val Thr Lys Asn Phe Pro Asn Lys Phe 595 600 605	1056
GAC TCC GAT AAT AAG GTT ATT CAG TTC GAA GTG AGC TGG CGC AAG ATT Asp Ser Asp Asn Lys Val Ile Gln Phe Glu Val Ser Trp Arg Lys Ile 610 615 620	1104
TCG ACG GCT ATT TAT GGC GAT GCC AAA AAC GGC GTA TTT AAC AAA GAT Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe Asn Lys Asp 625 630 635	1152
TAT GAC TTC GGT TTT GGC AAG GTT CGT CAG GTG AAA GAT TTG CAG ATG Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp Leu Gln Met 640 645 650	1200
GGT CTG CTG ATG TAC TTG GGC AAG CCG AAA TAA Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys 655 660	1233

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 465 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..462
- (D) OTHER INFORMATION: /product= "FGF 2 - Ile Mutation at Residue 116"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC	48
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly	
415 420 425	
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
430 435 440	
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC CAC CCC GAC GGC CGA GTT	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg His Pro Asp Gly Arg Val	
445 450 455	
GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT CAA	192
Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln	
460 465 470	
GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC CGT	240
Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg	
475 480 485 490	
TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT GTT	288
Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val	
495 500 505	
ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC AAT	336
Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn	
510 515 520	
ACT TAC ATA TCA AGG AAA TAC ACC AGT TGG TAT GTG GCA TTG AAA CGA	384
Thr Tyr Ile Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys Arg	
525 530 535	
ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA GCT	432
Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala	
540 545 550	

189

ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TAA
 Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
 555 560

465

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 465 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..462
- (D) OTHER INFORMATION: /product= "FGF 2 - Glu Mutation at Residue 119"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly 155 160 165 170	48
GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu 175 180 185	96
TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC CAC CCC GAC GGC CGA GTT Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg His Pro Asp Gly Arg Val 190 195 200	144
GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT CAA Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln 205 210 215	192
GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC CGT Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg 220 225 230	240
TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT GTT Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val 235 240 245 250	288
ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC AAT Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn 255 260 265	336
ACT TAC CGG TCA AGG GAA TAC ACC AGT TGG TAT GTG GCA TTG AAA CGA Thr Tyr Arg Ser Arg Glu Tyr Thr Ser Trp Tyr Val Ala Leu Lys Arg 270 275 280	384

190

ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA GCT 432
 Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala
 285 290 295

ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TAA 465
 Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
 300 305

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 465 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..462
- (D) OTHER INFORMATION: /product= "FGF 2 - Ala Mutation at Residue 120"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC 48
 Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
 155 160 165 170

GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG 96
 Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
 175 180 185

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC CAC CCC GAC GGC CGA GTT 144
 Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg His Pro Asp Gly Arg Val
 190 195 200

GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT CAA 192
 Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln
 205 210 215

GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC CGT 240
 Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg
 220 225 230

TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT GTT 288
 Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val
 235 240 245 250

ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC AAT 336
 Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn
 255 260 265

191

ACT TAC CGG TCA AGG AAA GCA ACC AGT TGG TAT GTG GCA TTG AAA CGA 384
 Thr Tyr Arg Ser Arg Lys Ala Thr Ser Trp Tyr Val Ala Leu Lys Arg
 270 275 280

ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA GCT 432
 Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala
 285 290 295

ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TAA 465
 Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
 300 305

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 465 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..462
- (D) OTHER INFORMATION: /product= "FGF 2 - Trp Mutation at Residue 123"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

ATG GCA GCA GGA TCA ATA ACA ACA TTA CCC GCC TTG CCC GAG GAT GGC 48
 Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
 155 160 165 170

GGC AGC GGC GCC TTC CCG CCC GGC CAC TTC AAG GAC CCC AAG CGG CTG 96
 Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
 175 180 185

TAC TGC AAA AAC GGG GGC TTC TTC CTG CGC CAC CCC GAC GGC CGA GTT 144
 Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg His Pro Asp Gly Arg Val
 190 195 200

GAC GGG GTC CGG GAG AAG AGC GAC CCT CAC ATC AAG CTT CAA CTT CAA 192
 Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln
 205 210 215

GCA GAA GAG AGA GGA GTT GTG TCT ATC AAA GGA GTG TGT GCT AAC CGT 240
 Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg
 220 225 230

TAC CTG GCT ATG AAG GAA GAT GGA AGA TTA CTG GCT TCT AAA TGT GTT 288
 Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val
 235 240 245 250

192

ACG GAT GAG TGT TTC TTT TTT GAA CGA TTG GAA TCT AAT AAC TAC AAT	336
Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn	
255 260 265	
ACT TAC CGG TCA AGG AAA TAC ACC AGT GCA TAT GTG GCA TTG AAA CGA	384
Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Ala Tyr Val Ala Leu Lys Arg	
270 275 280	
ACT GGG CAG TAT AAA CTT GGA TCC AAA ACA GGA CCT GGG CAG AAA GCT	432
Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala	
285 290 295	
ATA CTT TTT CTT CCA ATG TCT GCT AAG AGC TAA	465
Ile Leu Phe Leu Pro Met Ser Ala Lys Ser	
300 305	

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Protamine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TACATGCCAT GGCCAGGTAC AGATGCTGTC GCAGCCAGAG CCGGAGCAGA TATTACCGCC 60

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Protamine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GCAGCTCCGC CTCCTTCGTC TGCGACTTCT TTGTCTCTGG CGGTAATATC TGCTCCGGCT 60

193

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: /note= "Primer for Protamine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GACGAAGGAG GCGGAGCTGC CAGACACGGA GGAGAGCCAT GAGGTGCTGC CGCCCCAGGT 60

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: /note= "Primer for Protamine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

ATATATCCTA GGTTAGTGTC TTCTACATCT CGGTCTGTAC CTGGGGCGGC AGCACCTCA 59

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

CGTATCAGGC GGCCGCCGCC ATGGTGACCT CCATCACCCT GGACCTGGTG AACCCACCG 60

CCGGCC 66

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

TTGGGGTCCT TCACGTTGTT GCGGATCTTG TCCACGAAGG AGGAGTACTG GCCGGCGGTG 60
GGGTTCACC 69

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 65 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

AACAACGTGA AGGACCCCAA CCTGAAGTAC GCGGGCACCG ACATCGCCGT GATCGGCCCC 60
CCCTC 65

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 65 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

195

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GTGCCGCGGG AGGACTGGAA GTTGATGCGC AGGAATTCT CCTTGGAGGG GGGGCCGATC 60
ACGGC 65

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon
Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

CTCCCGCGGC ACCGTGTCCC TGGCCTGAA GCGCGACAAC CTGTACGTGG TGGCCTACCT 60
GGCCATGGAC AACAC 75

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon
Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

GCGGTCAGCT CGGCGGAGGT GATCTCGGAC TTGAAGTAGT AGGCGCGGTT CACGTTGGTG 60
TTGTCCATGG CCAGGTA 77

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 78 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

196

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GCCGAGCTGA CCGCCCTGTT CCCTGAGGCC ACCACCGCCA ACCAGAAGGC CCTGGAGTAC 60
ACCGAGGACT ACCAGTCC 78

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

AGCCCGAGCT CCTTGCGGGA CTTGTCGCCC TGGGTGATCT GGGCGTTCTT CTCGATGGAC 60
TGGTAGTCCT CGGTGT 76

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

TATAGAATTC CTCGGGCTGG GCATCGACCT GCTGCTGACC TTCATGGAGG CCGTGAACAA 60
GAAGGCCCGC GTGG 74

197

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CGGCGGTCAT CTGGATGGCG ATCAGCAGGA AGCGGGCCTC GTTCTTCACC ACGCGGGCCT 60
TCTTGTTT 68

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CGCCATCCAG ATGACCGCCG AGGTGGCCCG CTTCCGCTAC ATCCAGAACC TGGTGACCAA 60
GAACTTCCCC 70

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

198

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GGCGGATCCC AGCTGACCTC GAACTGGATC ACCTGTTGT CGGAGTCGAA CTTGTTGGGG 60
AAGTTCTTGG TCACCA 76

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon
Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

CCGGGATCCG TCAGCTGGCG CAAGATCTCC ACCGCCATCT ACGGCGACGC CAAGAACGGC 60
G 61

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 64 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon
Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GCACCTTGCC GAAGCCGAAG TCGTAGTCCT TGTGAACAC GCCGTTCTTG GCGTCGCCGT 60
AGAT 64

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

199

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TTCGGCTTCG GCAAGGTGCG CCAGGTGAAG GACCTGCAGA TGGGCCTGCT GATGTACC 58

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 52 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for Mammalian Codon Preferred Saporin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

TGAACGTGGC GGCCGCCTAC TTGGGCTTGC CCAGGTACAT CAGCAGGCCC AT 52

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for SAP-6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CATATGTGTG TCACATCAAT CACATTAGAT 30

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

200

(ix) FEATURE:

(D) OTHER INFORMATION: /note= "Primer for SAP-6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

CAGGTTTGGA TCCTTTACGT T

21

Claims

1. A pharmaceutical composition having the formula:
receptor-binding internalized ligand—nucleic acid binding domain—cytocide-encoding agent, wherein:
receptor-binding internalized ligand is a polypeptide reactive with a cell surface receptor;
nucleic acid binding domain binds to a nucleic acid, the domain being conjugated or fused to the receptor-binding internalized ligand;
cytocide-encoding agent is a nucleic acid molecule encoding a cytocide, the agent being bound to the nucleic acid binding domain; and wherein
the receptor-binding internalized ligand—nucleic acid binding domain—cytocide-encoding agent binds to the cell surface receptor and internalizes the cytocide-encoding agent in cells bearing the receptor.
2. A pharmaceutical composition having the formula:
receptor-binding internalized ligand—nucleic acid binding domain—prodrug-encoding agent, wherein:
receptor-binding internalized ligand is a polypeptide reactive with a cell surface receptor;
nucleic acid binding domain binds to a nucleic acid, the domain being conjugated or fused to the receptor-binding internalized ligand;
prodrug-encoding agent is a nucleic acid molecule encoding a prodrug, the agent being bound to the nucleic acid binding domain; and wherein
the receptor-binding internalized ligand—nucleic acid binding domain—prodrug-encoding agent binds to the cell surface receptor and internalizes the cytocide-encoding agent in cells bearing the receptor.
3. The composition of either of claims 1 or 2 wherein the receptor-binding internalized ligand is a polypeptide reactive with an FGF receptor.

4. The composition of either of claims 1 or 2 wherein the receptor-binding internalized ligand is selected from the group consisting of a polypeptide reactive with a VEGF receptor and a polypeptide reactive with an HBEGF receptor.

5. The composition of either of claims 1 or 2 wherein the receptor-binding internalized ligand is a cytokine.

6. The composition of claim 1 wherein the cytocide-encoding agent encodes a protein that inhibits protein synthesis.

7. The composition of claim 6 wherein the protein is a ribosome inactivating protein.

8. The composition of claim 7 wherein the ribosome inactivating protein is saporin.

9. The composition of claim 7 wherein the ribosome inactivating protein is gelonin.

10. The composition of claim 6 wherein the protein inhibits elongation factor 2.

11. The composition of claim 10 wherein the protein is diphtheria toxin.

12. The composition of claim 2 wherein the prodrug-encoding agent encodes HSV-thymidine kinase.

13. The composition of either of claims 1 or 2 wherein the growth factor is a polypeptide reactive with the FGF receptor and the nucleic acid binding domain is poly-L-lysine.

14. The composition of either of claims 1 or 2 wherein the nucleic acid binding domain is selected from the group consisting of helix-turn-helix motif proteins, homeodomain proteins, zinc finger motif proteins, steroid receptor proteins, leucine zipper motif proteins, helix-loop-helix motif proteins, and β -sheet motif proteins.

15. The composition of either of claims 1 or 2 wherein the nucleic acid binding domain is selected from the group consisting of AP-1, Sp-1, *rev*, GCN4, λ cro, λ cI, TFIIA, myoD, retinoic acid receptor, glucocosteroid receptor, SV40 large T antigen, and GAL4.

16. The composition of either of claims 1 or 2 wherein the nucleic acid binding domain is selected from the group consisting of poly-L-lysine, protamine, histone and spermine.

17. The composition of claim 1 wherein the nucleic acid binding domain binds a DNA molecule that encodes a ribosome inactivating protein.

18. The composition of claim 1 wherein the nucleic acid binding domain binds the coding region of saporin DNA.

19. The composition of claim 1 wherein the cytocide-encoding agent further comprises a tissue-specific promoter.

20. The composition of claim 2 wherein the prodrug-encoding agent further comprises a tissue-specific promoter.

21. The composition of either of claims 19 or 20 wherein the tissue-specific promoter is selected from the group consisting of alpha-crystalline, tyrosinase,

α -fetoprotein, prostate specific antigen, CEA, α -actin, VEGF receptor, *erbB-2*, *C-myc*, cyclin D, FGF receptor and gamma-crystalline promoter.

22. The composition of any one of claims 1-21, further comprising at least one linker that increases the serum stability or intracellular availability of the nucleic acid binding domain, the addition of said linker(s) resulting in the formula:

receptor-binding internalized ligand—(L)_q—nucleic acid binding domain-cytocide encoding agent or the formula:

receptor-binding internalized ligand—(L)_q—nucleic acid binding domain-prodrug encoding agent wherein:

L is at least one linker; and

q is 1 or more, such that the conjugate retains the ability to bind to a cell surface receptor and internalize the cytocide-encoding agent, and wherein the cytocide-encoding agent is bound to the nucleic acid binding domain.

23. The composition of claim 22 wherein the linker increases the flexibility of the conjugate.

24. The composition of claim 23 wherein the linker is selected from the group consisting of (Gly_mSer_p)_n, (Ser_mGly_p)_n and (AlaAlaProAla)_n in which n is 1 to 6, m is 1 to 6 and p is 1 to 4.

25. The composition of claim 24 wherein m is 4, p is 1 and n is 2 to 4.

26. The composition of claim 22 wherein the linker is a disulfide bond.

27. A therapeutically effective amount of a pharmaceutical composition according to any one of claims 1, 2 or 22, for use in the manufacture of a medicament for preventing excessive cell proliferation in the eye, comprising contacting the eye with a cell proliferation-inhibiting amount, wherein:

the inhibited cells are epithelial cells, endothelial cells, fibroblast cells or keratocytes; and

the excessive amount is an amount greater than that required to heal the surgical wound.

28. A therapeutically effective amount of a pharmaceutical composition according to any one of claims 1, 2 or 22, for use in the manufacture of a medicament for treating cancer, comprising contacting the cancer cells with an amount of the composition sufficient for inhibiting proliferation of the cancer cells.

29. A therapeutically effective amount of a pharmaceutical composition according to any one of claims 1, 2 or 22, for use in the manufacture of a medicament for treating smooth muscle cell hyperplasia, comprising contacting the smooth muscle cells with an amount of the composition sufficient for inhibiting hyperplasia of smooth muscle cells.

30. A pharmaceutical composition having the formula:

receptor-binding internalized ligand-cytocide-encoding agent nucleic acid binding domain, wherein:

receptor-binding internalized ligand is a polypeptide reactive with a cell surface receptor;

cytocide-encoding agent is a nucleic acid molecule encoding a cytocide, the agent being conjugated to the receptor-binding internalized ligand; and wherein the cytocide-encoding agent is bound to the nucleic acid binding domain; and wherein the receptor-binding internalized ligand-cytocide-encoding agent nucleic acid binding domain binds to the cell surface receptor and is internalized in cells bearing the receptor.

31. The composition of claim 30 wherein the nucleic acid binding domain is poly-L-lysine.

32. The composition of claim 30 wherein the receptor binding internalized ligand is a polypeptide reactive with an FGF receptor.

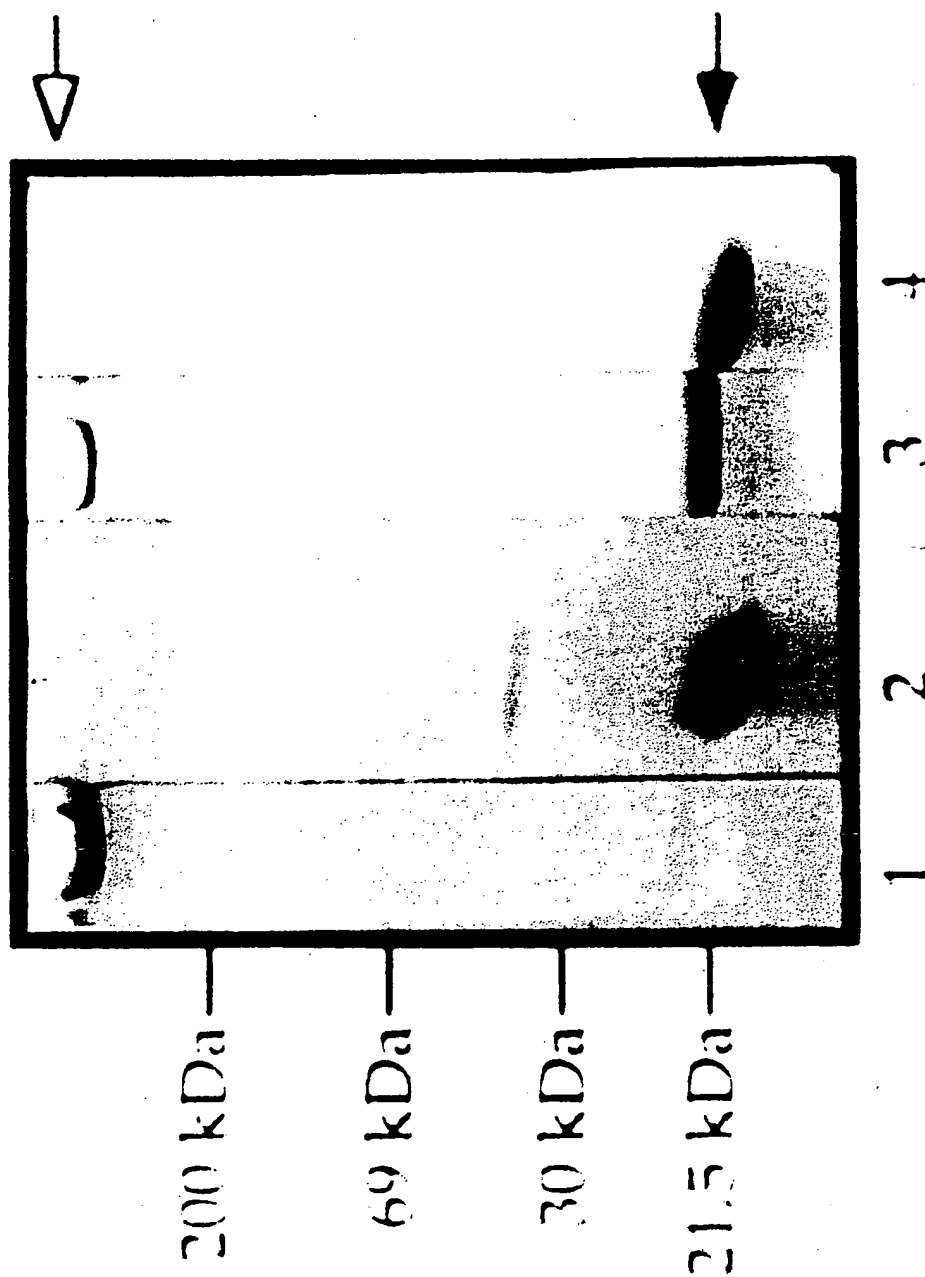
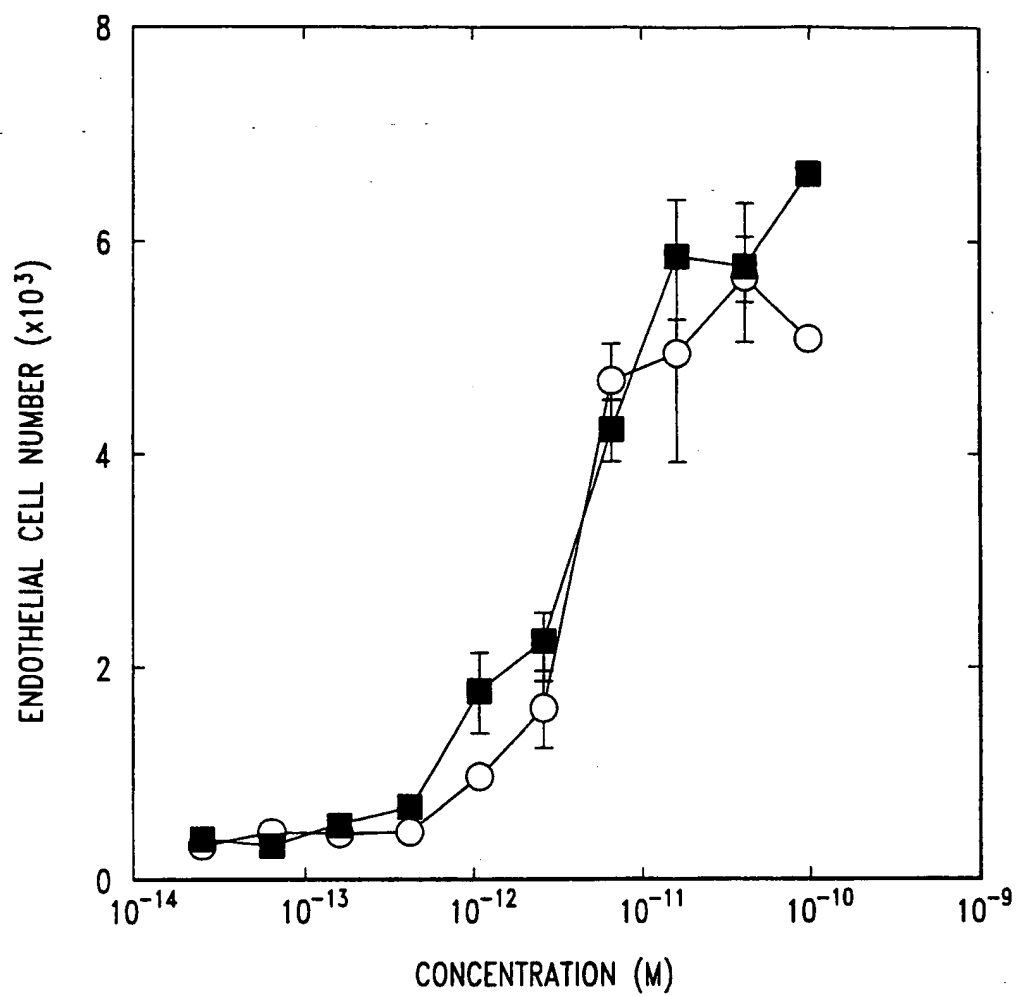


FIG. 1

2/13

*Fig. 2*

3/13

FIG. 3A

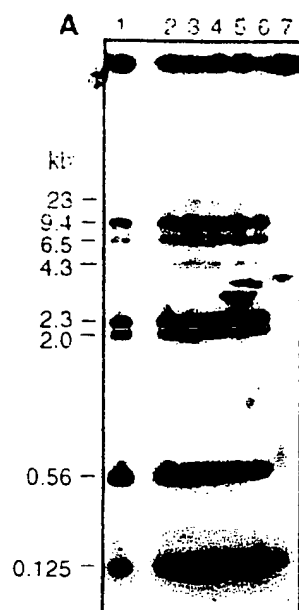


FIG. 3B

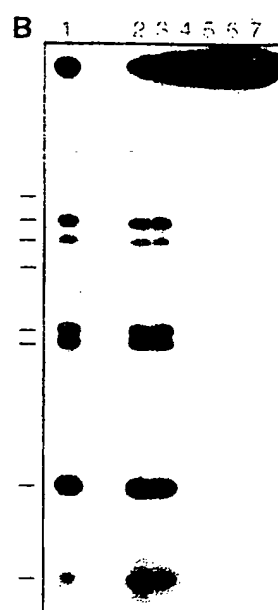


FIG. 3C

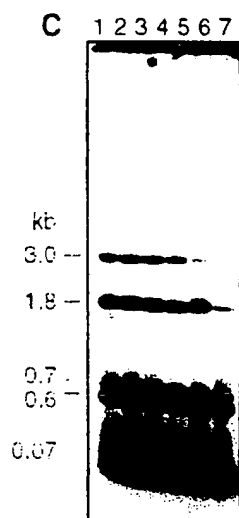


FIG. 3D

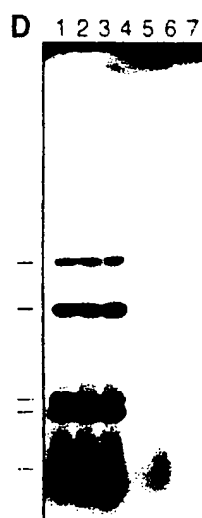


FIG. 3E

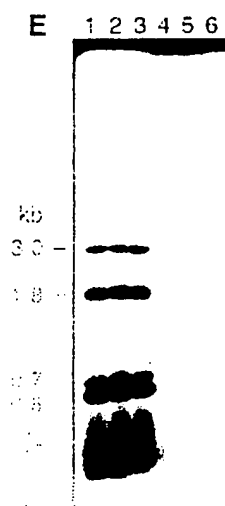
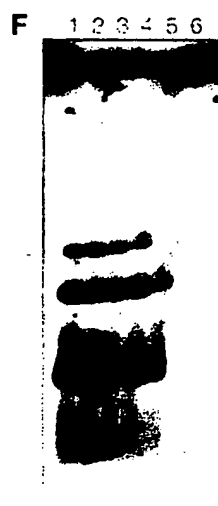
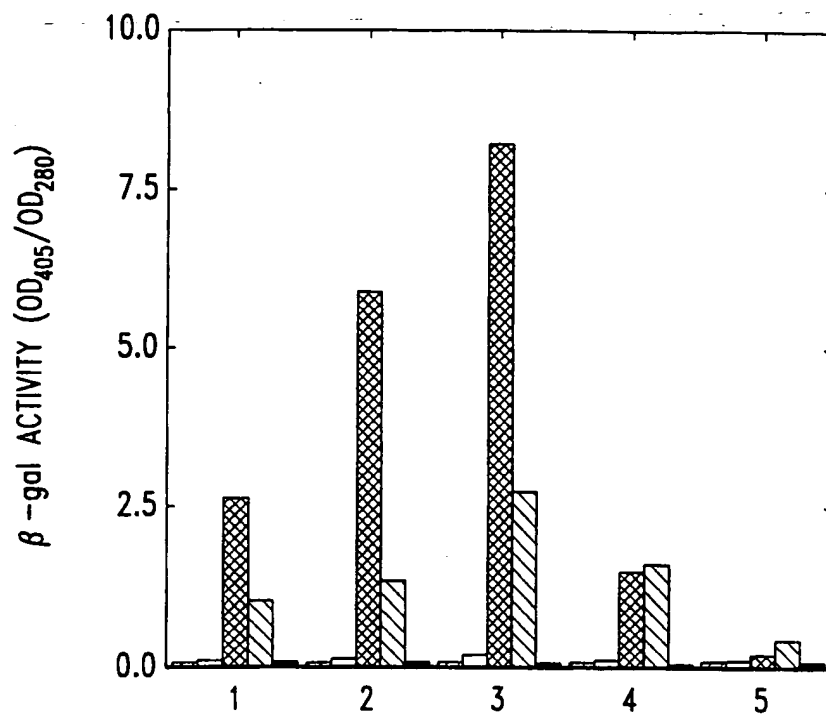


FIG. 3F



4/13

*Fig. 4*

5/13

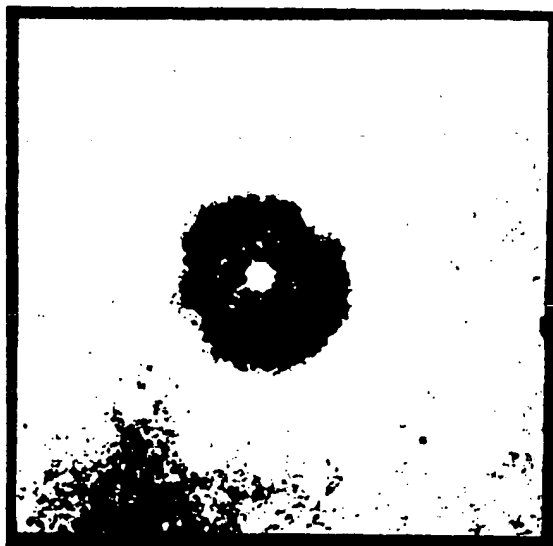


FIG. 5A

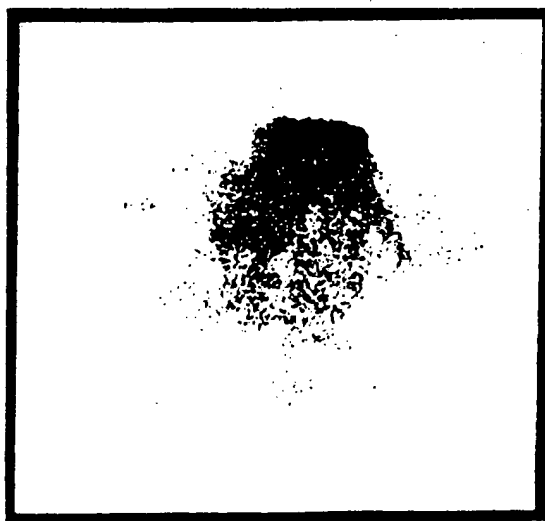
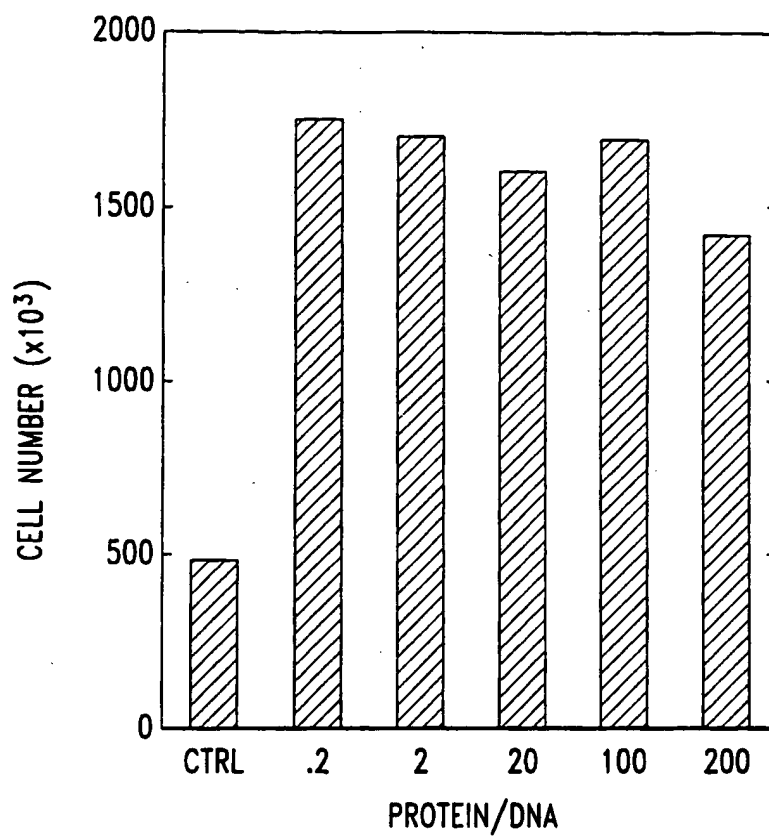
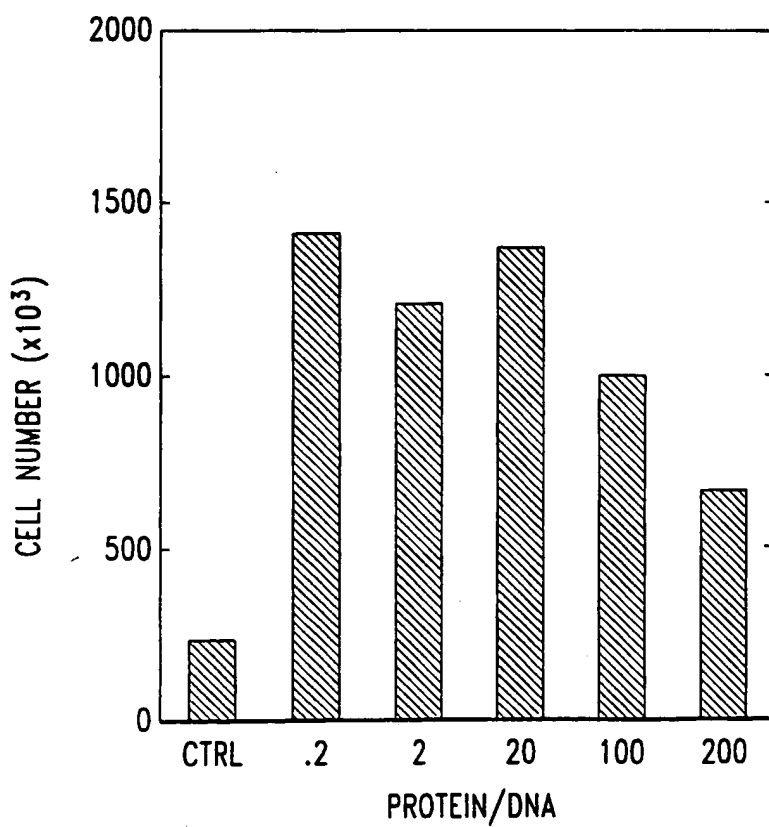
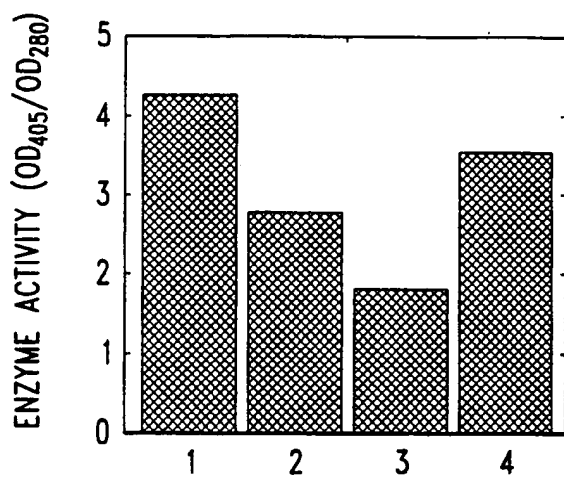
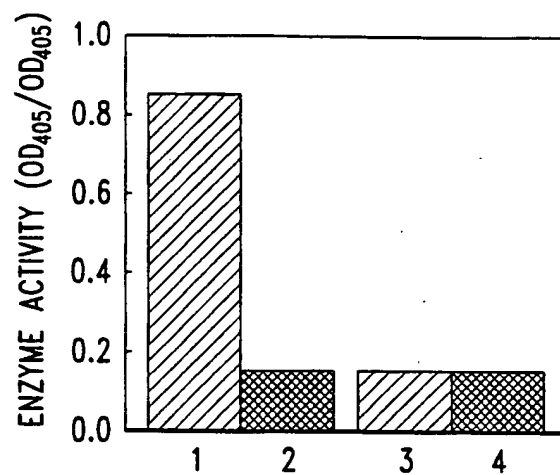
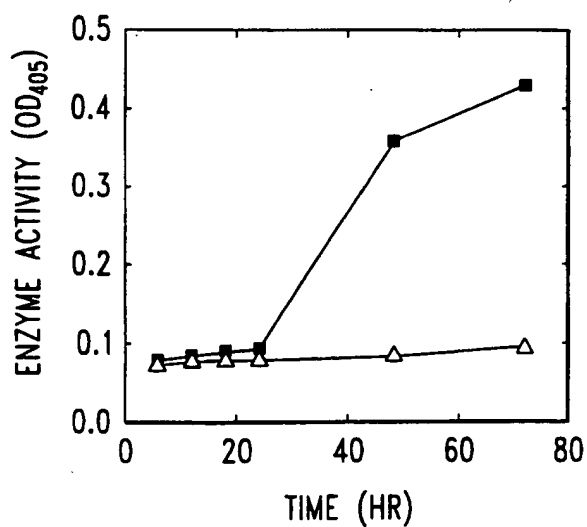
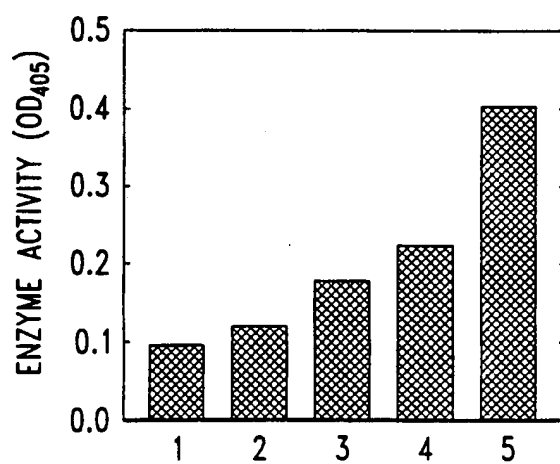


FIG. 5B

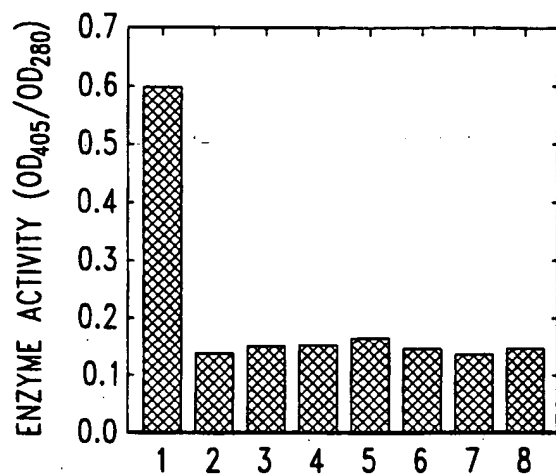
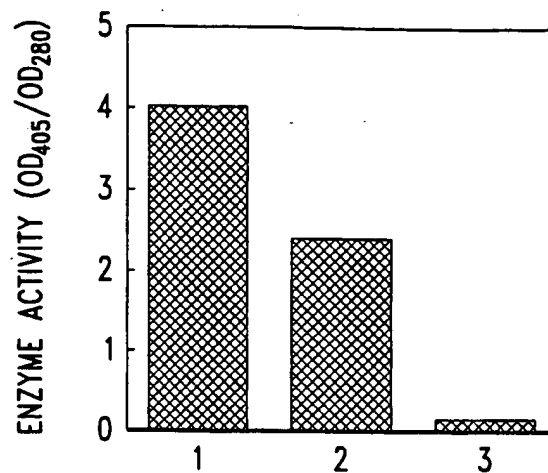
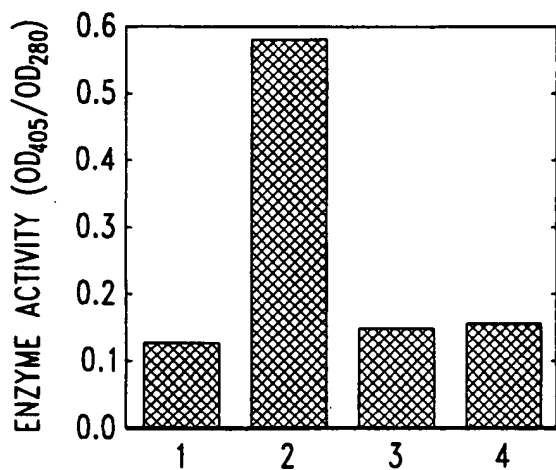
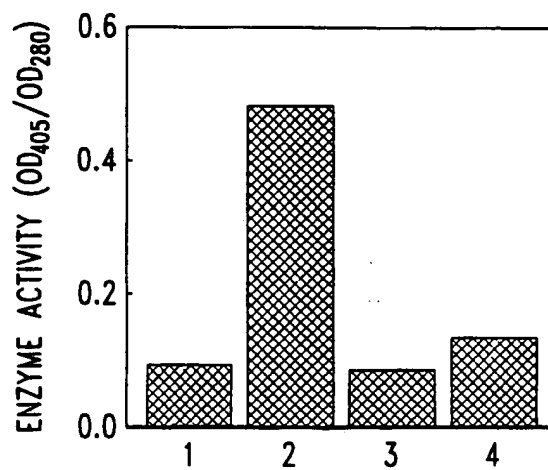
6/13

*Fig. 6A**Fig. 6B*

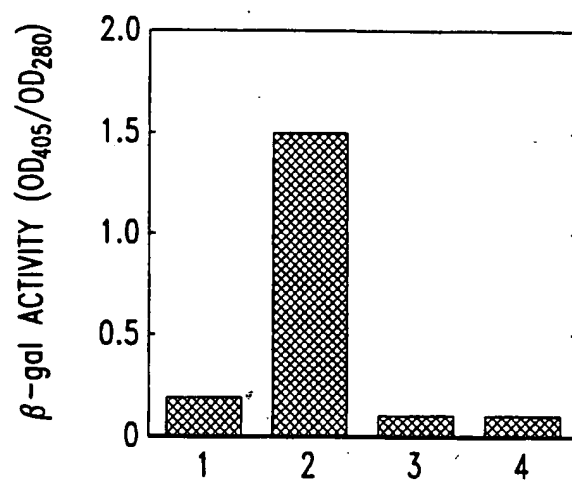
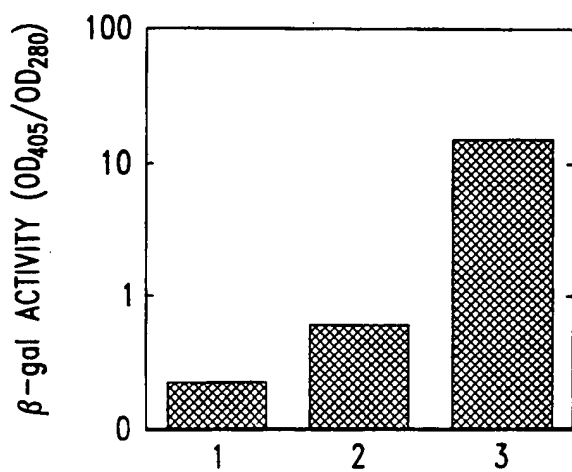
7/13

*Fig. 7A**Fig. 7B**Fig. 7C**Fig. 7D*

8/13

*Fig. 8A**Fig. 8B**Fig. 8C**Fig. 8D*

9/13

*Fig. 9A**Fig. 9B*

10/13

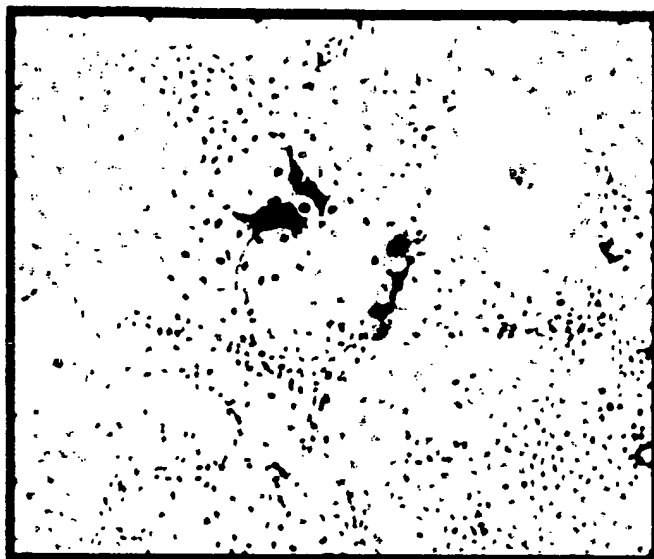
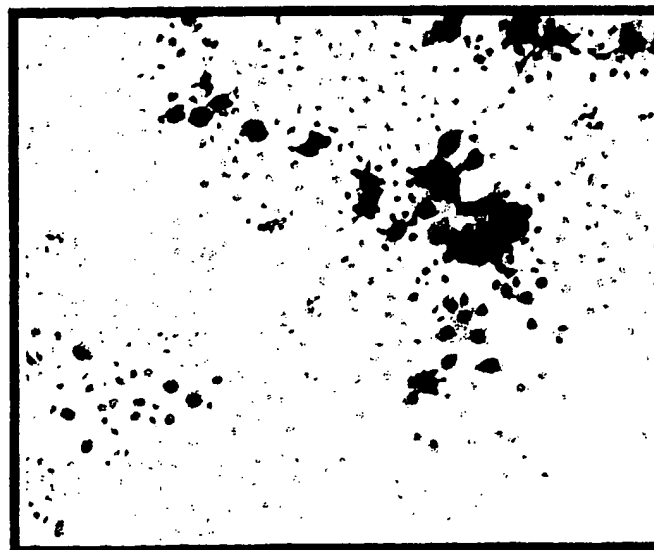


FIG. 9C



11/13

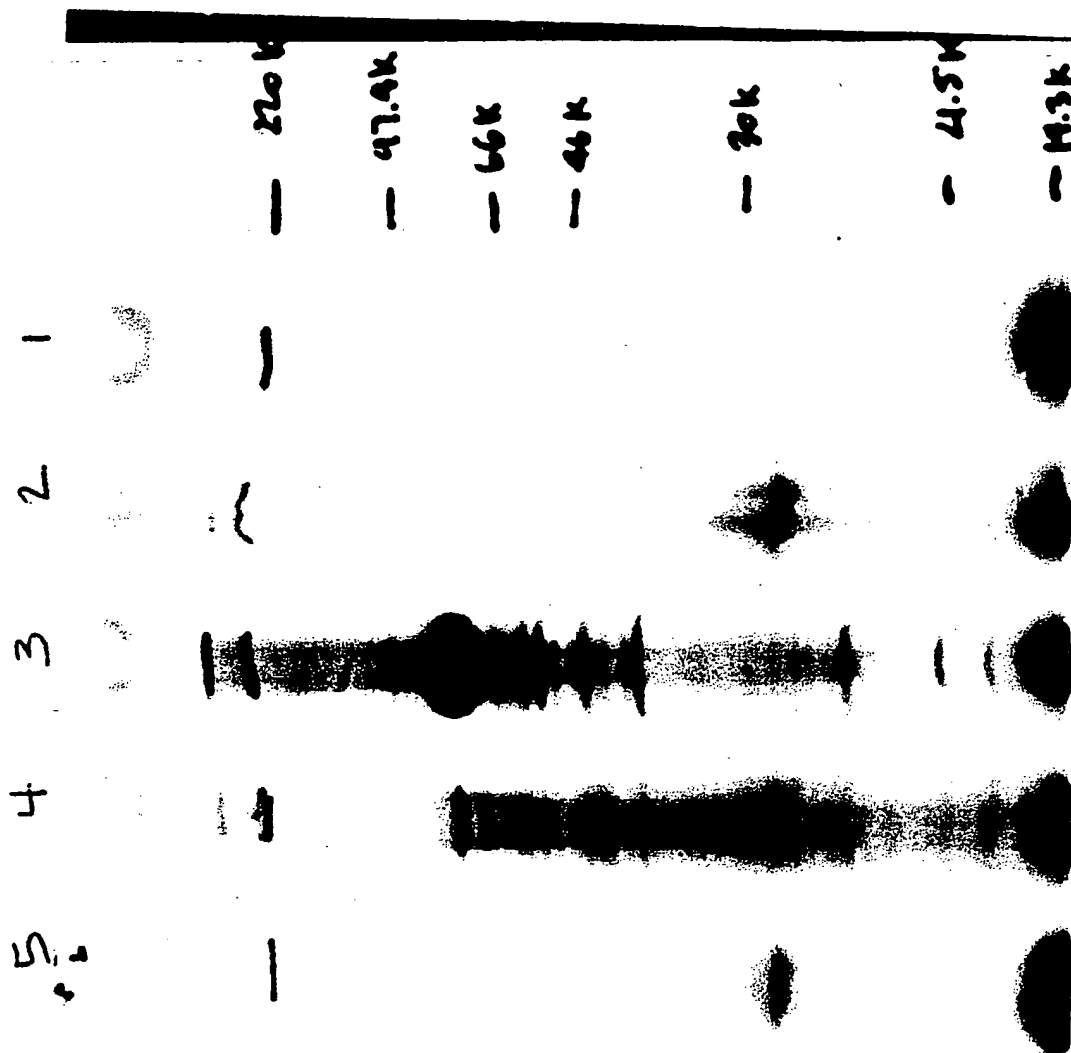
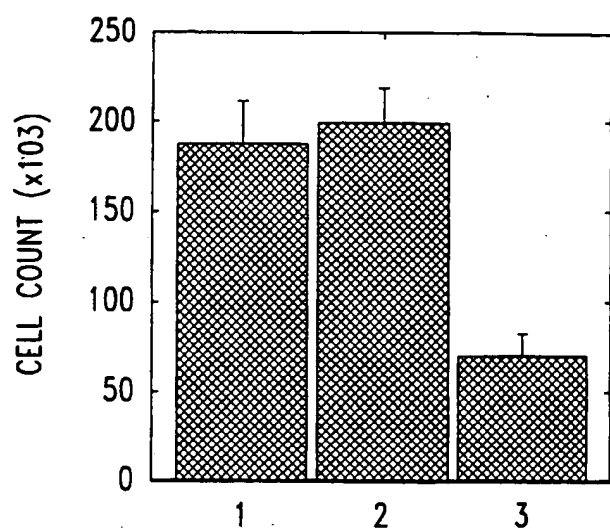
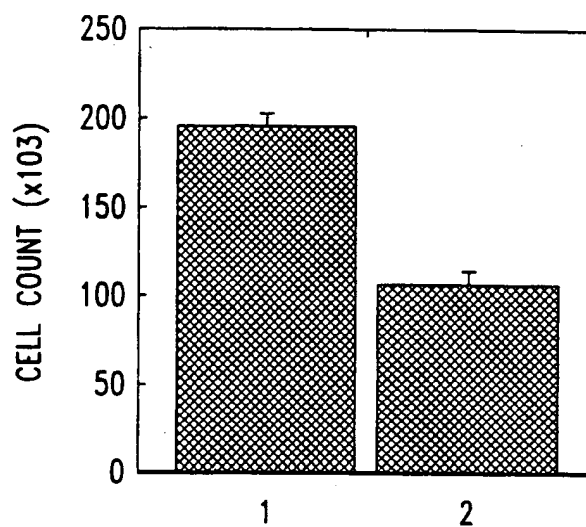
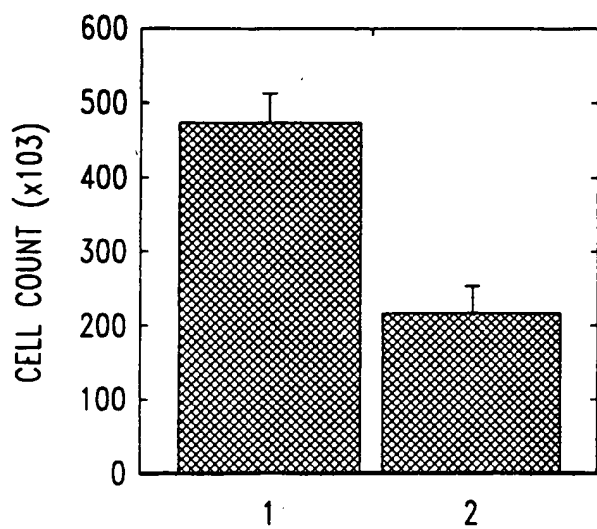
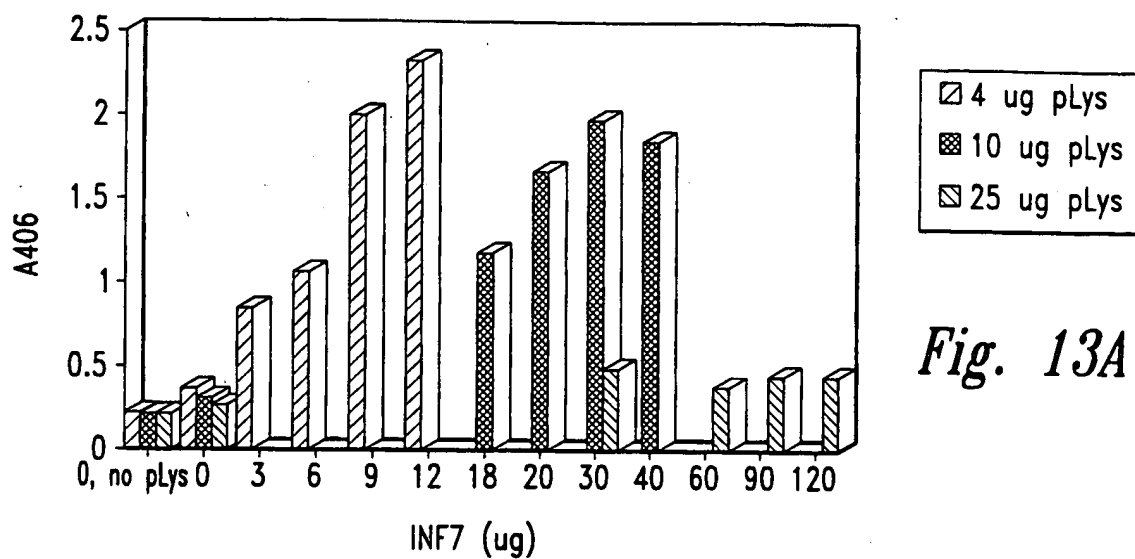
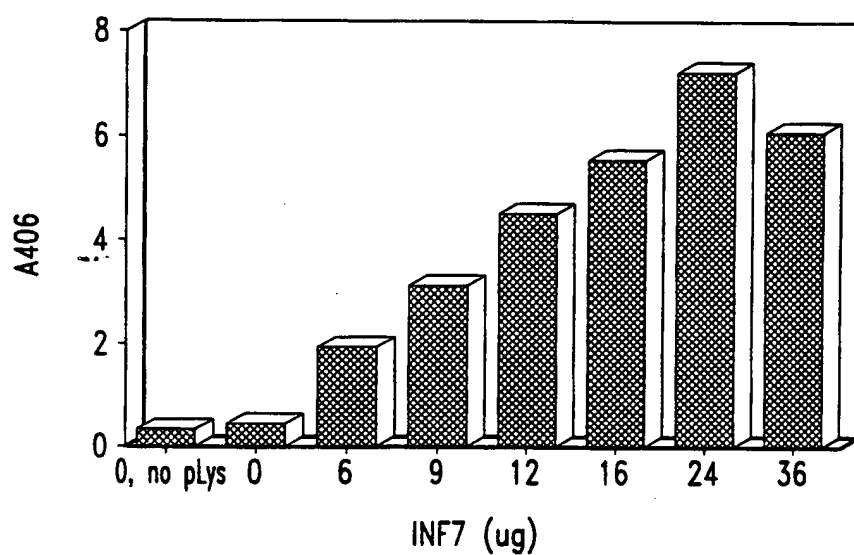
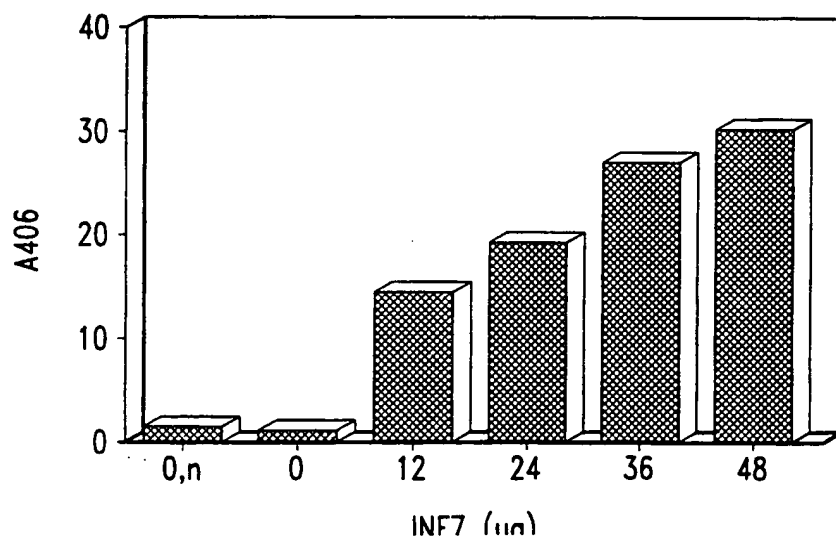


FIG. 10

12/13

*Fig. 11**Fig. 12A**Fig. 12B*

13/13

*Fig. 13A**Fig. 13B**Fig. 13C*

INTERNATIONAL SEARCH REPORT

International Application No

US 96/07164

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH .87TH ANNUAL MEETING, vol. 37, March 1996, WASHINGTON ,DC, pages 426-#2911, XP002016449 SOSNOWSKI, B.A. ET AL.: "TARGETING DNA THROUGH GROWTH FACTOR RECEPTORS." see the whole document ---	1-31
P,X	WO,A,95 24928 (PRIZM PHARMA INC) 21 September 1995 see page 9, line 24 - line 32 see page 6, line 31 - line 36 see page 7, line 6 - line 15; claims 1,6 see page 23, line 4 - line 8 see page 9, line 1 - line 8 see page 25, line 32 --- -/--	1-4,6-8, 13, 16-18, 21-32



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

21 October 1996

Date of mailing of the international search report

04.11.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
FAX (+31-70) 340-2040

Authorized officer

Ranta M

INTERNATIONAL SEARCH REPORT

International Application No.

US 96/07164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 25688 (WHITTIER INST. FOR DIABETES AND ;PRIZM PHARMACEUTICALS INC (US); LA) 23 December 1993 see claims 1,19-22 ---	1-32
Y,P	WO,A,95 28494 (TARGETED GENETICS CORP ;OVERELL ROBERT W (US); WEISSER KAREN E (US) 26 October 1995 see page 12, line 16 - line 29 see page 19, line 22 - line 32 see page 16, line 18 - line 27 see page 18, line 3 - line 16 X see page 38, line 1 see page 40, line 3 - line 19 ---	1-32
P,X	WO,A,96 08274 (PRIZM PHARMA INC) 21 March 1996 see page 21, line 22 - line 35 see page 26, line 11 - line 26 see page 29, line 9 - line 22 see page 52, line 16 - line 29 ---	1
P,X	WO,A,96 06641 (PRIZM PHARMA INC) 7 March 1996 see page 35, line 3 - line 8 ---	1
P,X	INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 37, no. 2, 15 February 1996, page S187 #885 XP002016450 B. A. SOSNOWSKI ET AL.: "RECEPTOR MEDIATED GENE DELIVERY THROUGH THE FGF RECEPTOR: APPLICATIONS IN THE EYE." & ANNUAL MEETING FORT LAUDERDALE,, 21 - 26 April 1996, FLORIDA, -----	1-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/07164

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-32
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Please see next page.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/210

In view of the large number of compounds, which are defined by the compounds mentioned in the claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, chapter III, paragraph 2.3)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

/US 96/07164

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9524928	21-09-95	AU-A- 2272495	03-10-95
WO-A-9325688	23-12-93	AU-A- 4635393	04-01-94
		CA-A- 2138038	23-12-93
		EP-A- 0646174	05-04-95
		JP-T- 7507926	07-09-95
WO-A-9528494	26-10-95	AU-A- 2387295	10-11-95
WO-A-9608274	21-03-96	AU-A- 3724495	29-03-96
		AU-A- 3374795	22-03-96
		WO-A- 9606641	07-03-96
WO-A-9606641	07-03-96	AU-A- 3374795	22-03-96
		AU-A- 3724495	29-03-96
		WO-A- 9608274	21-03-96